(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 April 2004 (29.04.2004)

PCT

(10) International Publication Number
WO 2004/035798 A2

(51) International Patent Classification[7]: C12N 15/82

(21) International Application Number:
PCT/EP2003/011658

(22) International Filing Date: 20 October 2003 (20.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02079408.7 18 October 2002 (18.10.2002) EP

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/035798 A2

(54) Title: IDENTIFICATION OF NOVEL E2F TARGET GENES AND USE THEREOF

(57) Abstract: The present invention concerns a method for altering characteristics of a plant. The invention describes the identification of genes that are upregulated or downregulated in transgenic plants overexpressing E2Fa/DPa and the use of such sequences to alter plant characteristics. A preferred way for altering characteristics of a plant comprises modifying expression of one or more nucleic acid sequences and or modifying level and/or activity of one or more proteins, which nucleic acids and/or proteins are essentially similar to any one or more of SEQ ID NO 1 to 2755. Some of the genes identified in the present invention have an E2Fa target consensus sequence in their 5' upstream region. The identified genes play a role in a variety of biological processes, such as DNA replication, cell wall biosynthesis, nitrogen and/or carbon metabolism, transcription factors etc.

IDENTIFICATION OF NOVEL E2F TARGET GENES AND USE THEREOF

The present invention concerns altering plant characteristics. More particularly, the present invention relates to identification of genes and proteins involved in E2Fa/DPa-mediated processes and further relates to use of such genes and proteins for altering characteristics in plants.

The present invention concerns a method for altering one or more plant characteristics, whereby the altered plant characteristic is selected from altered development, altered plant growth, altered, for example increased, plant yield and/or biomass, biochemistry, physiology, architecture, metabolism, survival capacity or stress tolerance by modifying expression of one or more of the genes according to the present invention and/or by modifying levels and/or activity of the proteins encoded by these genes. The present invention also concerns genetic constructs for performing the methods of the invention and to plants or plant parts obtainable by the methods of the present invention, which plants have altered characteristics compared to their otherwise isogenic counterparts. The invention also extends to recombinant nucleic acids and the use thereof in the methods according to the invention.

Growth, development and differentiation of higher organisms are controlled by a highly ordered set of events called the cell cycle (Morgan, 1997). Cell division and cell growth are operated by the cell cycle, which ensures correct timing and high fidelity of the different transition events involved. Cell cycle regulation at both G1→S and G2→M phase transitions depends on the formation of appropriate protein complexes and both transitions are believed to be the major control points in the cell cycle. The cell's decision to proliferate and synthesize DNA and ultimately to divide is made at the G1→S restriction point in late G1. Overcoming this point of no return requires the cell's competence to initiate DNA synthesis as well as the expression of S-phase genes. Transcription of S-phase-specific genes requires binding to the DNA of an E2F transcription factor. Dimerisation of E2F with DP is a prerequisite for high affinity binding to the E2F consensus DNA binding site (A/T)TT(G/C)(G/C)C(G/C)(G/C) (SEQ ID NO 2775), for example (TTT(C/G)(C/G)CGC), that can be found in the promoters of genes involved in DNA replication, repair, checkpoint control and differentiation (Ren et al., 2002; Weinmann et al., 2001; Kel et al., 2001). Variants of this consensus sequence as well as other locations of this consensus sequences are also found. The heterodimeric E2F/dimerization partner (DP) transcription factor also regulates the promoter activity of multiple genes, which are essential for DNA replication and cell cycle control (Helin, 1998; Müller and Helin, 2000). E2F transcription factors are critical effectors of the decision to pass the restriction point and to allow the cell to proceed in S-phase.

In the *Arabidopsis* genome, 3 E2F (E2Fa, E2Fb, and E2Fc) and 2 DP genes (DPa and DPb) are present (Vandepoele et al., 2002). The phenotypic analysis of plants overexpressing E2Fa and DPa was described recently (De Veylder et al., 2002). Microscopic analysis revealed that E2Fa/DPa overproducing cells underwent ectopic cell division or endoreduplication, depending on the cell type. Whereas extra cell divisions resulted in cells being smaller than those seen in the same tissues of control plants, extra endoreduplication caused formation of giant nuclei. RT-PCR demonstrated that expression levels of genes involved in DNA replication (CDC6, ORC1, MCM, DNA pol α) were strongly upregulated in plants overexpressing E2Fa and DPa (De Veylder et al., 2002).

The present invention provides genes having altered expression levels in plants overexpressing E2Fa and DPa relative to expression levels in corresponding wild type plants. Furthermore, the present invention provides means to modulate expression of these genes, which in turn allows for modulation of the biological processes that they control. The present invention provides methods to mimic E2F/DP level and/or activity by manipulating downstream factors involved in E2F/DP pathways. This strategy allows a fine-tuning of the effects of E2Fa/DPa. Whereas overexpression of E2Fa or DPa or both can be pleiotropic and/or can have pleiotropic effects, it is the invention provides methods to alter plant characteristics in a more controlled and targeted way, by using the E2F/DP target genes as defined by the present invention. Modulation of particular biological processes is now possible and may give rise to plants having altered characteristics, which may have particularly useful applications in agriculture and horticulture.

Therefore, according to the present invention, there is provided a method to alter one or more plant characteristics, comprising modifying, in a plant, expression of one or more nucleic acids and/or modifying level and/or activity of one or more proteins, which nucleic acids or proteins are essentially similar to any one of SEQ ID NO 1 to 2755, and wherein said one or more plant characteristics are altered relative to corresponding wild type plants.

The inventors designed a microarray experiment, comparing transcript levels of more than 4579 genes of wild type and transgenic *Arabidopsis* lines overexpressing E2Fa/DPa. Surprisingly, the inventors found that particular genes are up or down regulated in E2Fa-DPa overexpressing plants. The sequences which were at least 1.3 times upregulated or downregulated, are represented with their MIPS (Munich information center for protein sequences) accession number MATDB database http://mips.gsf.de/proj/thal/db/index.html) in Tables 4 and 5. Sequences which were at least 2-fold upregulated or 2-fold downregulated are shown in Tables 1 and 2, respectively. Further classification of these genes according to their function is provided in Tables 1 and 2. Promoter analysis of these genes allowed for the

identification of genes under the direct control of E2Fa and/or DPa proteins and genes that are indirectly controlled by the E2Fa/DPa complex. Examples of mechanisms for such indirect control include, (i) recognition by E2F/DP of other sequence elements that diverge from the consensus recognition site; (ii) possible association of E2F/DP with other DNA binding proteins capable of recognizing other DNA elements; and (iii) sequential transcription activation of a first gene capable of regulating transcription of a second gene. It is to be understood that having an E2F target sequence is not a prerequisite to be regulated by E2F.

The gene that corresponds to the sequence deposited under the MIPS database accession number At1g57680 is an example of a gene, which is likely to be indirectly controlled by the E2Fa/DPa complex. This gene is of unknown function. It was surprising to find this unknown gene and the other genes of Tables 1, 2, 4 and 5 to be involved in E2Fa/DPa controlled processes. The genes according to the present invention are represented herein with their nucleic acid sequence and corresponding amino acid sequence as set forth in SEQ ID NO 1 to 2755.

Preferably expression and/or level and/or activity of one of the genes and/or proteins according to any of SEQ ID NO 1 to 2755 is modified. Alternatively expression and/or level and/or activity of one or more of those genes and/or proteins is modified. According to a further embodiment one or more gene/and or proteins of the same functional category as presented in Table 1 or Table 2, are modified.

The term "modifying expression" relates to altering level (increasing expression or decreasing expression) or altering the time or altering the place of expression of a nucleic acid. The term "modified" as used herein is used interchangeably with "altered" or "changed".
Modified expression (or level or activity) of a sequence essentially similar to any one of SEQ ID NO 1 to 2755 encompasses changed expression (or level or activity) of a gene product, namely a polypeptide, in specific cells or tissues. The changed expression, activity and/or levels are changed compared to expression, activity and/or levels of the gene or protein essentially similar to any one of SEQ ID NO 1 to 2755 acid in corresponding wild-type plants. The changed gene expression may result from changed expression levels of an endogenous gene essentially similar to any one of SEQ ID NO 1 to 2755 acid and/or may result from changed expression levels of a gene essentially similar to SEQ ID NO 1 to 2755 acid previously introduced into a plant. Similarly, changed levels and/or activity of a protein essentially similar to any one of SEQ ID NO 1 to 2755 acid may be due to changed expression of an endogenous nucleic acid/gene and/or due to changed expression of nucleic acid/gene previously introduced into a plant.

Modified expression of a gene/nucleic acid and/or increasing or decreasing activity and/or levels of a gene product may be effected, for example, by chemical means and/or recombinant means.

Advantageously, modified expression of a nucleic acid according to the invention and/or modified activity and/or levels of a protein according to the invention may be effected by chemical means, i.e. by exogenous application of one or more compounds or elements capable of modifying activity and/or levels of the protein and/or capable of modifying expression of a nucleic acid/gene according to the invention. The term "exogenous application" as defined herein is taken to mean the contacting or administering of a suitable compound or element to plant cells, tissues, organs or to the whole organism. The compound or element may be exogenously applied to a plant in a form suitable for plant uptake (such as through application to the soil for uptake via the roots, or in the case of some plants by applying directly to the leaves, for example by spraying). The exogenous application may take place on wild-type plants or on transgenic plants that have previously been transformed with a nucleic acid/gene according to the present invention or with another transgene.

Suitable compounds or elements include proteins or nucleic acids according to SEQ ID NO 1 to 2755 or proteins or nucleic acids essentially similar to SEQ ID NO 1 to 2755. Essentially similar proteins or nucleic acids are, homologues, derivatives or active fragments of these proteins and/or portions or sequences capable of hybridizing with these nucleic acids. The exogenous application of yet other compounds or elements capable of modifying levels of factors that directly or indirectly activate or inactivate a protein according to the present invention will also be suitable in practicing the invention. These compounds or elements also include antibodies that can recognize or mimic the function of the proteins according to the present invention. Such antibodies may comprise "plantibodies", single chain antibodies, IgG antibodies and heavy chain camel antibodies, as well as fragments thereof. Additionally or alternatively, the resultant effect may also be achieved by the exogenous application of an interacting protein or activator or an inhibitor of the gene/gene product according to the present invention. Additionally or alternatively, the compound or element may be a mutagenic substance, such as a chemical selected from any one or more of: N-nitroso-N-ethylurea, ethylene imine, ethyl methanesulphonate and diethyl sulphate. Mutagenesis may also be achieved by exposure to ionising radiation, such as X-rays or gamma-rays or ultraviolet light. Methods for introducing mutations and for testing the effect of mutations (such as by monitoring gene expression and/or protein activity) are well known in the art.

Therefore, according to one aspect of the present invention, there is provided a method for altering plant characteristics, comprising exogenous application of one or more compounds or elements capable of modifying expression of a gene and/or capable of modifying activity and/or levels of a protein according to the present invention.

Additionally or alternatively, and according to a preferred embodiment of the present invention, modified of expression of a nucleic acid and/or modified of activity and/or levels of a protein, wherein these nucleic acids or proteins are essentially similar to any of SEQ ID NO 1 to 2755, may be effected by recombinant means. Such recombinant means may comprise a direct and/or indirect approach for modifying expression of a nucleic acid and/or for modifying activity and/or levels of a protein.

Therefore there is provided by the present invention, a method to alter plant characteristics, comprising modifying gene expression and/or protein levels and/or protein activity, which modification may be effected by recombinant means and/or by chemical means and wherein said gene and/or protein are essentially similar to any one of SEQ ID NO 1 to 2755.

An indirect recombinant approach may comprise for example introducing, into a plant, a nucleic acid capable of increasing or decreasing activity and/or levels of the protein in question (a protein essentially similar to any one of SEQ ID NO 1 to 2755) and/or capable of increasing or decreasing expression of the gene in question (a gene essentially similar to any one of SEQ ID NO 1 to 2755). Examples of such nucleic acids to be introduced into a plant, are nucleic acids encoding transcription factors or activators or inhibitors that bind to the promoter of a gene or that interact with a protein essentially similar to any one of SEQ ID NO 1 to 2755. Methods to test these types of interactions and methods for isolating nucleic acids encoding such interactors include yeast one-hybrid or yeast two-hybrid screens.

Also encompassed by an indirect approach for modifying activity and/or levels of a protein according to the present invention and/or expression of a gene according to the present invention, is the provision of a regulatory sequence, or the inhibition or stimulation of regulatory sequences that drive expression of the native gene in question or of the transgene in question. Such regulatory sequences may be introduced into a plant. For example, the nucleic acid introduced into the plant is a promoter, capable of driving the expression of the endogenous gene essentially similar to any one of SEQ ID NO 1 to 2755.

A further indirect approach for modifying activity and/or levels and/or expression of a gene or protein according to the present invention in a plant encompasses modifying levels in a plant of

a factor able to interact with the protein according to the present invention. Such factors may include ligands of the protein according to the present invention. Therefore, the present invention provides a method for altering characteristics of a plant, when compared to the corresponding wild-type plants, comprising modifying expression of a gene coding for a protein which is a natural ligand of a protein essentially similar to any one of SEQ ID NO 1 to 2755. Furthermore, the present invention also provides a method to alter one or more plant characteristics relative to corresponding wild-type plants, comprising modifying expression of a gene coding for a protein which is a natural target/substrate of a protein essentially similar to SEQ ID NO 1 to 2755.

A direct and more preferred approach to alter one or more plant characteristics, comprises introducing into a plant a nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755, wherein said nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755 is any one of SEQ ID NO 1 to 2755 or a portion thereof or sequences capable of hybridizing therewith and which nucleic acid preferably encodes a protein essentially similar to any one of SEQ ID NO 1 to 2755, which protein essentially similar to any one of SEQ ID NO 1 to 2755 is any one of SEQ ID NO 1 to 2755 or a homologue, derivative or active fragment thereof. The nucleic acid may be introduced into a plant by, for example, transformation.

In the context of the present invention the term "modifying expression" and modifying level and/or activity encompasses "enhancing or decreasing". Methods for obtaining enhanced or increased expression of genes or gene products are well documented in the art and are for example overexpression driven by a strong promoter, the use of transcription enhancers or translation enhancers. The term "overexpression" of a gene refers to expression patterns and/or expression levels of said gene normally not occurring under natural conditions. Ectopic expression can be achieved in a number of ways including operably linking of a coding sequence encoding said protein to an isolated homologous or heterologous promoter in order to create a chimeric gene.

Alternatively and/or additionally, increased expression of a gene or increased activities and/or levels of a protein in a plant cell, is achieved by mutagenesis. For example these mutations can be responsible for the changed control of the gene, resulting in more expression of the gene, relative to the wild-type gene. Mutations can also cause conformational changes in a protein, resulting in more activity and/or levels of the protein.

Examples of decreasing expression of a gene are also well documented in the art and include for example: downregulation of expression by anti-sense techniques, RNAi techniques, small

interference RNAs (siRNAs), microRNA (miRNA), etc. Therefore according to a particular aspect of the invention, there is provided a method to alter characteristics of plants, including technologies that are based on for example the synthesis of antisense transcripts, complementary to the mRNA of a gene essentially similar to any one of SEQ ID NO 1 to 2755.

Another method for downregulation of gene expression or gene silencing comprises use of ribozymes, for example as described in WO9400012 (Atkins *et al.*), WO9503404 (Lenee *et al.*), WO0000619 (Nikolau *et al.*), WO9713865 (Ulvskov *et al.*) and WO9738116 (Scott *et al.*).

Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by gene silencing strategies as described among others in the documents WO9836083 (Baulcombe and Angell), WO9853083 (Grierson *et al.*), WO9915682 (Baulcombe *et al.*) or WO9953050 (Waterhouse *et al.*).

Expression of an endogenous gene may also be reduced if the endogenous gene contains a mutation. Such a mutant gene may be isolated and introduced into the same or different plant species in order to obtain plants having altered characteristics. Also dominant negative mutants of a nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755 can be introduced in the cell to decrease the level/and or activity of the endogenous corresponding protein.

Other methods to decrease the expression of a nucleic acid and/or activity and/or level of proteins essentially similar to any one of SEQ ID NO 1 to 2755 in a cell encompass, for example, the mechanisms of transcriptional gene silencing, such as the methylation of the promoter of a gene according to the present invention.

Modifying expression of the gene also encompasses altered transcript level of the gene. Altered transcript levels of a gene can be sufficient to induce certain phenotypic effects, for example via the mechanism of cosuppression. Here the overall effect of overexpression of a transgene is that there is less level and/or activity in the cell of the protein, which is encoded by the native gene showing homology to the introduced transgene.

Cosuppression is accomplished by the addition of coding sequences or parts thereof in a sense orientation into the cell. Therefore, according to one aspect of the present invention, the characteristics of a plant may be changed by introducing into a plant an additional copy (in full or in part) of a gene essentially similar to any one of SEQ ID NO 1 to 2755 already present in a host plant. The additional gene may silence the endogenous gene, giving rise to a phenomenon known as co-suppression.

According to the invention, "nucleic acid" or the "gene" essentially similar to any one of SEQ ID NO 1 to 2755 in a plant may be the wild type gene, i.e. native or endogenous or heterologous, i.e. derived from another individual plant or plant species. The gene (transgene) may be substantially modified from its native form in composition and/or genomic environment through deliberate human manipulation. This transgene can be introduced into a host cell by transformation techniques. Also expression of the native genes can be modified by introduction in the plant of regulatory sequences capable of altering expression of the native gene, as described above.

The term "modifying activity" relates to enhancing, decreasing or altering time or place of activity of a protein or polypeptide. According to the invention, the "protein" or the "polypeptide" may be the wild type protein, i.e. native or endogenous, or alternatively, the protein may be heterologous, i.e. derived from another individual or species.

The term "essentially similar to" in relation to a protein of the present invention as used herein includes variants such as homologues, derivatives and functional fragment thereof. The term "essentially similar to" in relation to a gene includes variants such as at least a part of the gene in question; a complement of the gene; RNA, DNA, a cDNA or a genomic DNA corresponding to the protein or gene; a variant of the gene due to the degeneracy of the genetic code; a family member of the gene or protein; an allelic variant of the gene or protein; and different splice variant of the gene or protein and variants that are interrupted by one or more intervening sequences. Advantageously, nucleic acids or proteins essentially similar to nucleic acids and the proteins according to any of SEQ ID NO 1 to 2755 may be used in the methods of the present invention. These variant nucleic acids and variant amino acids are described further below.

Any variant of a particular protein according to the present invention is a variant, which upon construction of a phylogenetic tree with that particular protein, tends to cluster around the particular protein which is any one of SEQ ID NO 1 to 2755. Such a phylogenetic tree can be constructed with alignments of amino acid sequences or with nucleic acid sequences. A person skilled in the art could readily determine whether any variant in question falls within the definition of a "a nucleic acid or protein essentially similar to any one of SEQ ID NO 1 to 2755". Hereto the man skilled in the art would use known techniques and software for the making of such phylogenetic trees, such as a GCG, EBI or CLUSTAL package, or Align X, using default parameters. Advantageously, the methods according to the present invention may also be practised using such variants.

Any variant suitable for use in the methods according to the invention may readily be determined using routine techniques, such as by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the fragment to be tested for functionality.

A first example of such variants are "homologues" of the proteins of the present invention, which homologues encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or additions relative to the protein in question and having similar biological and functional activity as an unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984).

The homologues useful in the method according to the invention may have at least 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48% or 50% sequence identity or similarity (functional identity) to the unmodified protein, alternatively at least 52%, 54%, 56%, 58% or 60% sequence identity or similarity to an unmodified protein, or alternatively at least 62%, 64%, 66%, 68% or 70% sequence identity or similarity to an unmodified protein. Typically, the homologues have at least 72%, 74%, 76%, 78% or 80% sequence identity or similarity to an unmodified protein, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89% sequence identity or similarity, further preferably at least 90%, 91%, 92%, 93% or 94% sequence identity or similarity to an unmodified protein, further preferably at least 95% 96%, 97%, 98% or 99% sequence identity or similarity to an unmodified protein. This % identity can be calculated using the Gap program in the WISCONSIN PACKAGE version 10.0-UNIX from Genetics Computer Group, Inc based on the method of Needleman and Wunsch (J. Mol. Biol. 48:443-453 (1970)) using the set of default parameters for pairwise comparison (for amino acid sequence comparison: Gap Creation Penalty = 8, Gap Extension Penalty = 2; for nucleotide sequence comparison: Gap Creation Penalty = 50; Gap Extension Penalty = 3).

The percentage of identity can also be calculated by using other alignment program well known in the art. For example, the percentage of identity can be calculated using the program needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 5.5 software package, using the parameters (for example GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

These above-mentioned analyses for comparing sequences may be done on full-length sequences but additionally or alternatively the calculation of sequence identity or similarity can be based on a comparison of certain regions such as conserved domains.

The identification of such domains, would also be well within the realm of a person skilled in the art and involves, for example, running a computer readable format of the nucleic acids of the present invention in alignment software programs, scanning publicly available information on protein domains, conserved motifs and boxes. This type of information on protein domains is available in the PRODOM (http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html), PIR (http://pir.georgetown.edu/), INTERPRO (http://www.ebi.ac.uk/interpro/) or pFAM (http://pfam.wustl.edu/) database. Sequence analysis programs designed for motif searching can be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs would include but are not limited to: MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 2.2) can be found in version 10.0 of the GCG package; or on the Internet site http://www.sdsc.edu/MEME/meme. SIGNALSCAN version 4.0 information is available on the Internet site http://biosci.cbs.umn.edu/software/sigscan.html. GENESCAN can be found on the Internet site http://gnomic.stanford.edu/GENESCANW.html.

As mentioned above the nucleic acid suitable for practising the methods of the present invention can be wild type (native or endogenous). Alternatively, the nucleic acid may be derived from another (or the same) species, which gene is introduced into the plant as a transgene, for example by transformation. The nucleic acid may thus be derived (either directly or indirectly (if subsequently modified)) from any source provided that the nucleic acid, when expressed in a plant, leads to modified expression of a nucleic acid/gene or modified activity and/or levels of a protein essentially similar to SEQ ID NO 1 to 2755. The nucleic acid may be isolated from a microbial source, such as bacteria, yeast or fungi, or from a plant, algae, insect, or animal (including human) source. Methods for the search and identification of other homologues of the proteins of the present invention, or for nucleic acid sequences encoding homologues of proteins of the present invention would be well known to person skilled in the art. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information.

Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "paralogous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins used in the methods according to the invention.

A preferred homologue is a homologue obtained from a plant, whether from the same plant species or different. The nucleic acid may be isolated from a dicotyledonous species, preferably from the family *Brassicaceae*, further preferably from *Arabidopsis thaliana*.

Suitable homologues for use in the methods of the present invention have been identified in the genomes of rice and maize. These homologues are represented by their Genbank accession numbers in Table 1 and 2. Other homologues, especially orthologues from other plant species, are identifiable by methods well known by a person skilled in the art. *In silico*, methods involve running sequence alignment programs with the sequence of interest as mentioned above. *In vivo* methods involve the DNA encoding the protein of interest and are for example PCR techniques using degenerated primers designed based on the sequence of interest, which is any one essentially similar to any one of SEQ ID NO 1 to 2755, or hybridisation techniques with at least part of the sequence of interest.

"Substitutional variants" of a protein are those in which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about 1-20 residues.

"Insertional variants" of a protein are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, $(histidine)_6$-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein are characterized by the removal of one or more amino acids from the protein. Amino acid variants of a protein may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen *in vitro* mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

The term "derivatives" of a protein according to the present invention are those peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise substitutions, deletions or additions of naturally and non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the protein as deposited under the accession numbers presented in Table 1, 2, 4 and 5. "Derivatives" of a protein of the present invention encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein of the present invention.

Another variant useful in the methods of the present invention is an active fragment of a protein essentially similar to any one of SEQ ID NO 1 to 2755. The expression "functional fragment" in relation to a protein refers to a fragment that encompasses contiguous amino acid residues of said protein, and that has retained the biological activity of said naturally-occurring protein. For example, useful fragments comprise at least 10 contiguous amino acid residues of a protein essentially similar to any one of SEQ ID NO 1 to 2755. Other preferred fragments are fragments of these proteins starting at the second or third or further internal methionin residues. These fragments originate from protein translation, starting at internal ATG codons.

Advantageously, the method according to the present invention may also be practiced using fragments of DNA or of a nucleic acid sequence. The term "DNA fragment or DNA segment"

refers to a piece of DNA derived or prepared from an original (larger) DNA molecule. The term is not restrictive to the content of the DNA fragment or segment. For example, the DNA fragment or segments can comprise many genes, with or without additional control elements or may contain spacer sequences. A functional fragment refers to a piece of DNA derived or prepared from an original (larger) DNA molecule, which DNA portion, when introduced and expressed in a plant, gives plants having altered characteristics. The fragments may be made by making one or more deletions and/or truncations to the nucleic acid sequence. Techniques for introducing truncations and deletions into a nucleic acid are well known in the art.

Another example of variants useful in the methods of the present invention, encompasses nucleic acid sequences capable of hybridising with a nucleic acid sequence as presented in any one of SEQ ID NO 1 to 2755 or a nucleic acid encoding a protein as presented in any one of SEQ ID NO 1 to 2755.

The term "hybridisation" as defined herein is the process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. Tools in molecular biology relying on such a process include the polymerase chain reaction (PCR; and all methods based thereon), subtractive hybridisation, random primer extension, nuclease S1 mapping, primer extension, reverse transcription, cDNA synthesis, differential display of RNAs, and DNA sequence determination. The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to e.g. a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridisation, plaque hybridisation, *in situ* hybridisation and microarray hybridisation. In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration and hybridisation buffer composition. High stringency conditions for hybridisation include high temperature and/or low salt concentration (salts include NaCl and Na_3-citrate) and/or the inclusion of formamide in the hybridisation buffer and/or lowering the concentration of compounds such as SDS (detergent) in the hybridisation buffer and/or exclusion of compounds such as dextran sulphate or

polyethylene glycol (promoting molecular crowding) from the hybridisation buffer. Conventional hybridisation conditions are described in, for example, Sambrook (2001), but the skilled craftsman will appreciate that numerous different hybridisation conditions can be designed in function of the known or the expected homology and/or length of the nucleic acid sequence. With specifically hybridising is meant hybridising under stringent conditions. Specific conditions for "specifically hybridising" are for example: hybridising under stringent conditions such as a temperature of 60°C followed by washes in 2XSSC, 0.1XSDS, and 1X SSC, 0.1X SDS. Depending on the source and concentration of the nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed, such as medium stringency conditions. Examples of medium stringency conditions include 1-4x SSC / 0.25% w/v SDS at ≥ 45°C for 2-3 hours. Sufficiently low stringency hybridisation conditions are particularly preferred to isolate nucleic acids heterologous to the DNA sequences of the invention defined supra. Elements contributing to heterology include allelism, degeneration of the genetic code and differences in preferred codon usage. The stringency conditions may start low and be progressively increased until there is provided a hybridising nucleic acid, as defined hereinabove. Elements contributing to heterology include allelism, degeneration of the genetic code and differences in preferred codon usage.

Another variant useful in the methods for altering growth characteristics encompasses a nucleic acid sequence which is an alternative splice variant of a gene of the present invention (deposited in the MIPS database under the accession numbers as presented in Tables 1, 2, 4 or 5). The term "alternative splice variant" as used herein encompasses variants in which introns and selected exons have been excised, replaced or added. Such splice variants may be found in nature or can be manmade. For example, introns or exons can be excised, replaced or added such that the mRNA has altered expression (e.g. seed-preferred expression), or altered response to specific signals). Preferred variants will be ones in which the biological activity of the proteins of the present invention remains unaffected, which can be achieved by selectively retaining functional segments of the proteins. Methods for making such splice variants are well known in the art.

Another example of a variant useful to alter plant characteristics, is an allelic variant of a gene essentially similar to any one of SEQ ID NO 1 to 2755. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these isolated natural alleles in the methods according to the invention. Allelic variants encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp. SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms. Allellic variation can also

be created artificially, such as for example by the techniques of EMS mutagenesis. Typically such variants are created with the purpose of breeding the altered plant characteristic according to the present invention in a plant. Alternatively, naturally mutated alleles are the subject of such selection and breeding programmes, wherein the allele capable of conferring altered plant characteristics to the plant are selected and plants containing such allele are used for further breeding the trait.

Accordingly, the present invention provides a method for altering plant characteristics, using a splice variant or an allelic variant of a nucleic acid sequence according to any one of SEQ ID NO 1 to 2755.

The term "plant characteristic" means any characteristic of a plant, plant cell or plant tissue described hereafter. These characteristics encompass but are not limited to, characteristics of plant development, plant growth, yield, biomass production, plant architecture, plant biochemistry, plant physiology, metabolism, survival capacity, stress tolerance and more. DNA synthesis, DNA modification, endoreduplication, cell cycle, cell wall biogenesis, transcription regulation, signal transduction, storage lipid mobilization, photosynthesis and more.

The term "altering plant characteristics" as used herein encompasses any change in said characteristic such as increase, decrease or change in time or place. According to a preferred embodiment of the invention, altering a plant characteristics encompasses improving the plant characteristic, such as for example increasing the plant characteristic (e.g. yield), or accelerating the characteristic (e.g. growth rate).

"Growth" refers to the capacity of the plant or of plant parts to expand and increase in biomass. Altered growth refers amongst others to altered growth rate, cycling time, the size, expansion or increase of the plant. Additionally and/or alternatively, growth characteristics may refer to cellular processes comprising, but not limited to, cell cycle (entry, progression, exit), cell division, cell wall biogenesis and/or DNA synthesis, DNA modification and/or endoreduplication.

"Yield" refers to the harvestable part of the plant. "Biomass" refers to any part of the plants. These terms also encompass an increase in seed yield, which includes an increase in the biomass of the seed (seed weight) and/or an increase in the number of (filled) seeds and/or in the size of the seeds and/or an increase in seed volume, each relative to corresponding wild-type plants. An increase in seed size and/or volume may also influence the composition of seeds. An increase in seed yield could be due to an increase in the number and/or size of

flowers. An increase in yield may also increase the harvest index, which is expressed as a ratio of the total biomass over the yield of harvestable parts, such as seeds.

"Plant development" means any cellular process of a plant that is involved in determining the developmental fate of a plant cell, in particular the specific tissue or organ type into which a progenitor cell will develop. Typical plant characteristics according to the present invention are therefore characteristics relating to cellular processes relevant to plant development such as for example, morphogenesis, photomorphogenesis, shoot development, root development, vegetative development, reproductive development, stem elongation, flowering, regulatory mechanisms involved in determining cell fate, pattern formation, differentiation, senescence, time of flowering and/or time to flower.

"Plant architecture", as used herein refers to the external appearance of a plant, including any one or more structural features or a combination of structural features thereof. Such structural features include the shape, size, number, position, colour, texture, arrangement, and patternation of any cell, tissue or organ or groups of cells, tissues or organs of a plant, including the root, stem, leaf, shoot, petiole, trichome, flower, petal, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre, fruit, cambium, wood, heartwood, parenchyma, aerenchyma, sieve element, phloem or vascular tissue, amongst others.

The term "stress tolerance" is understood as the capability of better survival and/or better performing in stress conditions such as environmental stress, which can be biotic or abiotic. Salinity, drought, heat, chilling and freezing are all described as examples of conditions which induce osmotic stress. The term "environmental stress" as used in the present invention refers to any adverse effect on metabolism, growth or viability of the cell, tissue, seed, organ or whole plant which is produced by a non-living or non-biological environmental stressor. More particularly, it also encompasses environmental factors such as water stress (flooding, water logging, drought, dehydration), anaerobic (low level of oxygen, CO2 etc.), aerobic stress, osmotic stress, salt stress, temperature stress (hot/heat, cold, freezing, frost) or nutrients deprivation, pollutants stress (heavy metals, toxic chemicals), ozone, high light, pathogen (including viruses, bacteria, fungi, insects and nematodes) and combinations of these. Biotic stress is stress as a result of the impact of a living organism on the plant. Examples are stresses caused by pathogens (virus, bacteria, nematodes insects etc.). Another example is stress caused by an organism, which is not necessarily harmful to the plant, such as the stress caused by a symbiotic or an epiphyte. Accordingly, particular plant characteristics according to the present invention encompass early vigour, survival rate, stress tolerance.

Field-grown plants almost always experience some form of stress, albeit mild, and therefore the terms “growth”, “yield” “biomass production” or “biomass” do not distinguish the performance of plants under non-stressed from performance under stress conditions. Advantageously, the effects of the invention on growth and yield are expected to occur under both severe and mild stress conditions (i.e. under stressed and non-stressed conditions).

Characteristics related to "plant physiology" encompass characteristics of functional processes of a plant, including developmental processes such as growth, expansion and differentiation, sexual development, sexual reproduction, seed set, seed development, grain filling, asexual reproduction, cell division, dormancy, germination, light adaptation, photosynthesis, leaf expansion, fiber production, secondary growth or wood production, amongst others; responses of a plant to externally-applied factors such as metals, chemicals, hormones, growth factors, environment and environmental stress factors (e.g. anoxia, hypoxia, high temperature, low temperature, dehydration, light, day length, flooding, salt, heavy metals, amongst others), including adaptive responses of plants to said externally-applied factors. Particular plant physiology characteristics which are altered according to the methods of the present invention encompass altered storage lipid mobilization, photosynthesis, transcription regulation and signal transduction.

Characteristics related to "plant biochemistry" are to be understood by those skilled in the art to refer to the metabolic characteristics. “Metabolism” as used in the present invention is interchangeable with biochemistry. Metabolism and/or biochemistry encompass catalytic or assimilation or other metabolic processes of a plant, including primary and secondary metabolism and the products thereof, including any element, small molecules, macromolecules or chemical compounds, such as but not limited to starches, sugars, proteins, peptides, enzymes, hormones, growth factors, nucleic acid molecules, celluloses, hemicelluloses, calloses, lectins, fibres, pigments such as anthocyanins, vitamins, minerals, micronutrients, or macronutrients, that are produced by plants. Preferably, the methods of the present invention are used to change the nitrogen or carbon metabolism.

As shown in Tables 1 and 2, several of the E2Fa-DPa target genes identified have an E2F recognition sequence in their promoter and most of these genes are involved in DNA replication. Therefore, provided by a particular embodiment of the present invention is a method as described above to influence DNA synthesis and DNA replication. The secondary induced genes, which are the genes not having the E2F target consensus sequence in their promoter region, encode proteins involved in cell wall biosynthesis, transcription, signal

transduction, or have an unknown function. Surprisingly, a large number of metabolic genes were modified as well, mainly genes involved in nitrate assimilation or metabolism and carbon metabolism.

The putative direct E2Fa-DPa target genes as identified by the presence of an E2F-DP-binding site, mainly belong to the group of genes involved in DNA synthesis, whereas the secondary induced genes are mainly linked to nitrogen assimilation and carbohydrate metabolism. Therefore, it is elucidated by the present invention that enhanced levels of E2Fa-DPa in plants have an impact on expression levels of genes involved in nitrogen assimilation and/or carbon metabolism. The experimental data suggest that in E2Fa/DPa overexpressing plants there is a drain of nitrogen to the nucleotide synthesis pathway causing a decreased synthesis of other nitrogen compounds such as amino acids and storage proteins. Corresponding to these findings, the inventors found that the level of endoreduplication of E2Fa-DPa transgenic plants depends on the amount of nitrogen available in the medium. Also, these data suggest that the growth arrest observed upon E2Fa/DPa expression results at least from a nitrogen drain to the nucleotide synthesis pathway, causing a decreased synthesis of other nitrogen components, such as amino acids and storage components.

As purine and pyrimidine bases are nitrogen-rich, the induction of nitrogen assimilation genes in the E2Fa-DPa transgenic plants is a mechanism to supply enough nitrogen for nucleotide biosynthesis. Most likely this drain of nitrogen from essential biosynthetic pathways to the nucleotide biosynthesis pathway has its effects on many aspects of plant metabolism, as can be seen from the reduction of expression of vegetative storage protein genes and genes involved in amino-acid biosynthesis.

Therefore a particular aspect of the invention is the use of genes involved in carbon and/or nitrogen metabolism or allocation, for altering nitrogen and carbon metabolism and/ or to alter the balance between carbon and nitrogen or to reallocate carbon and/or nitrogen or to alter the composition of components containing carbon and nitrogen. The elucidation of genes that are able to shift the nitrogen assimilation from one biological process to another biological process is important for many applications. These genes can for example be used to alter the nitrogen composition of nitrogen-containing compounds in a cell, such as nicotinamide-containing molecules, amino acid, nucleic acid, chlorophyll or any other metabolites. Also within the scope of the present inventions are these altered components obtainable by the methods of the present invention, with altered balance between carbon and nitrogen.

Therefore, according to the present invention, there is provided a method as described above, wherein said altered metabolism comprises altered nitrogen and/or carbon metabolism.

In a particular embodiment, said carbon metabolism comprises the processes of carbon fixation, photosynthesis and photorespiration. In another embodiment, said nitrogen metabolism comprises nitrogen fixation or the reallocation of nitrogen residues from the pool of amino acids into the pool of nucleic acids or vice versa.

Microarray analysis of E2Fa-DPa overexpressing lines, as herein described, identified a cross-talking matrix between DNA replication, nitrogen assimilation and photosynthesis. It has been described previously that there is a link between carbon:nitrogen availability and growth, storage lipid mobilization and photosynthesis (Martin T. (2002)). Therefore according to the present invention there is provided, a method as described above, wherein said altered plant characteristic comprises altered storage lipid mobilization and/or photosynthesis.

The microarray studies elucidated for the first time particular genes that are upregulated and particular genes that are downregulated in a plant cell overexpressing E2Fa/DPa, many of which were of unknown function. It is now disclosed how to use these genes and/or proteins for altering plant characteristics.

According to a preferred embodiment, recombinant means are used to alter plant characteristics. More preferably, one or more of the genes essentially similar to any of SEQ ID NO 1 to 2755 is introduced into a plant as a transgene. Accordingly, the present invention provides a recombinant nucleic acid comprising:

(a) one or more nucleic acid sequences essentially similar to any one of SEQ ID NO 1 to 2755; optionally operably linked to

(b) a regulatory sequence; and optionally operably linked to

(c) a transcription termination sequence.

This recombinant nucleic acid is suitable for altering plant growth characteristics.

Constructs useful in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells.

The genetic construct can be an expression vector wherein said nucleic acid sequence is operably linked to one or more control sequences allowing expression in prokaryotic and/or eukaryotic host cells.

The methods according to the present invention may also be practised by introducing into a plant at least a part of a (natural or artificial) chromosome (such as a Bacterial Artificial Chromosome (BAC)), which chromosome contains at least a gene/nucleic acid according to the present invention, optionally together with one or more related gene family members or genes belonging to the same functional group as for example the functional groups presented in Table 1 or 2. Therefore, according to a further aspect of the present invention, there is provided a method to alter plant characteristics, comprising introduction into a plant at least a part of a chromosome comprising at least a gene/nucleic, which gene/nucleic is essentially similar to any one of SEQ ID NO 1 to 2755.

In a particular embodiment of the present invention said regulatory sequence is a plant-expressible promoter. In a further embodiment of the invention the promoter is a constitutive promoter, such as the GOS2 promoter, the ubiquitin promoter, the actin promoter. In another embodiment of the invention the promoter is a promoter active in the meristem or in dividing cells, such as, but not limited to the cdc2 promoter, RNR promoter, MCM3 promoter. Alternatively, the regulatory element as mentioned above can be a translational enhancer, or a transcriptional enhancer that is used to enhance expression of a gene according to the present invention.

The term "Regulatory sequence" refers to control DNA sequences, which are necessary to affect expression of coding sequences to which they are operably linked. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoters, ribosomal binding sites, and terminators. In eukaryotes generally control sequences include promoters, terminators and enhancers or silencers. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components and which determines when, how much and where a specific gene is expressed. Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences derived from a classical eukaryotic genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. The term "promoter" also

includes the transcriptional regulatory sequences of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or a -10 box transcriptional regulatory sequences.

The term "promoter" is also used to describe a synthetic or fusion molecule or derivative, which confers; activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. "Promoter" is a DNA sequence generally described as the 5'-region of a gene, located proximal to the start codon. The transcription of an adjacent DNA segment is initiated at the promoter region. In the context of the present invention, the promoter preferably is a plant-expressible promoter sequence. Promoters, however, that also function or solely function in non-plant cells such as bacteria, yeast cells, insect cells and animal cells are not excluded from the invention. By "plant-expressible" is meant that the promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a plant cell, tissue or organ, preferably a monocotyledonous or dicotyledonous plant cell, tissue, or organ.

Preferably, the nucleic acid sequence capable of modulating expression of a gene encoding an E2F target protein is operably linked to a constitutive promoter or a tissue specific promoter. The term "constitutive" as defined herein refers to a promoter that is active predominantly in at least one tissue or organ and predominantly at any life stage of the plant. Preferably the promoter is active predominantly but not exclusively throughout the plant
Additionally and/or alternatively, the nucleic acid of the present invention may be operably linked to a tissue-specific promoter. The term "tissue-specific" promoter as defined herein refers to a promoter that is active predominantly but not exclusively in at least one tissue or organ.

Examples of preferred promoters useful for the methods of the present invention are presented in Table I, II, III and IV.

Table I:

Exemplary constitutive promoters for use in the performance of the present invention

GENE SOURCE	EXPRESSION PATTERN	REFERENCE
Actin	constitutive	McElroy *et al,* Plant Cell, 2: 163-171, 1990
CAMV 35S	constitutive	Odell *et al,* Nature, 313: 810-812, 1985
CaMV 19S	constitutive	Nilsson *et al., Physiol. Plant. 100*:456-462, 1997
GOS2	constitutive	de Pater *et al,* Plant J Nov;2(6):837-44, 1992
ubiquitin	constitutive	Christensen *et al,* Plant Mol. Biol. 18: 675-689, 1992

rice cyclophilin	constitutive	Buchholz *et al,* Plant Mol Biol. 25(5): 837-43, 1994
maize H3 histone	constitutive	Lepetit *et al,* Mol. Gen. Genet. 231:276-285, 1992
actin 2	constitutive	An *et al,* Plant J. 10(1); 107-121, 1996

Table II:

Exemplary seed-preferred promoters for use in the performance of the present invention

GENE SOURCE	EXPRESSION PATTERN	REFERENCE
seed-specific genes	seed	Simon, *et al., Plant Mol. Biol. 5:* 191, 1985; Scofield, *et al., J. Biol. Chem. 262:* 12202, 1987.; Baszczynski, *et al., Plant Mol. Biol. 14:* 633, 1990.
Brazil Nut albumin	seed	Pearson, *et al.*, Plant Mol. Biol. 18: 235-245, 1992.
legumin	seed	Ellis, *et al.*, Plant Mol. Biol. 10: 203-214, 1988.
glutelin (rice)	seed	Takaiwa, *et al.*, Mol. Gen. Genet. 208: 15-22, 1986; Takaiwa, *et al.*, FEBS Letts. 221: 43-47, 1987.
zein	seed	Matzke et al Plant Mol Biol, 14(3):323-32 1990
napA	seed	Stalberg, *et al, Planta 199:* 515-519, 1996.
wheat LMW and HMW glutenin-1	endosperm	Mol Gen Genet 216:81-90, 1989; NAR 17:461-2, 1989
wheat SPA	seed	Albani *et al,* Plant Cell, 9: 171-184, 1997
wheat a, b and g-gliadins	endosperm	EMBO 3:1409-15, 1984
barley *ltr1* promoter	endosperm	
barley B1, C, D, hordein	endosperm	Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996
barley DOF	endosperm	Mena *et al,* The Plant Journal, 116(1): 53-62, 1998
blz2	endosperm	EP99106056.7
synthetic promoter	endosperm	Vicente-Carbajosa *et al., Plant J. 13:* 629-640, 1998.
rice prolamin NRP33	endosperm	Wu *et al,* Plant Cell Physiology 39(8) 885-889, 1998
rice -globulin Glb-1	endosperm	Wu *et al,* Plant Cell Physiology 39(8) 885-889, 1998
rice OSH1	embryo	Sato *et al,* Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996
rice alpha-globulin REB/OHP-1	endosperm	Nakase *et al.* Plant Mol. Biol. 33: 513-522, 1997
rice ADP-glucose PP	endosperm	Trans Res 6:157-68, 1997
maize ESR gene family	endosperm	Plant J 12:235-46, 1997
sorgum gamma-kafirin	endosperm	PMB 32:1029-35, 1996

KNOX	embryo	Postma-Haarsma *et al*, Plant Mol. Biol. 39:257-71, 1999
rice oleosin	embryo and aleuron	Wu *et at*, J. Biochem., 123:386, 1998
sunflower oleosin	seed (embryo and dry seed)	Cummins, *et al.*, Plant Mol. Biol. 19: 873-876, 1992

Table III:

Exemplary flower-specific promoters for use in the performance of the invention

Gene source	Expression pattern	REFERENCE
AtPRP4	flowers	http://salus.medium.edu/mmg/tierney/html
chalene synthase (chsA)	flowers	Van der Meer, *et al., Plant Mol. Biol. 15*, 95-109, 1990.
LAT52	anther	Twell *et al* Mol. Gen Genet. 217:240-245 (1989)
apetala-3	flowers	

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Table IV:

Alternative rice promoters for use in the performance of the invention

PRO #	*gene*	expression
PRO0001	Metallothionein Mte	transfer layer of embryo + calli
PRO0005	putative beta-amylase	transfer layer of embryo
PRO0009	putative cellulose synthase	weak in roots
PRO0012	lipase (putative)	
PRO0014	transferase (putative)	
PRO0016	peptidyl prolyl cis-trans isomerase (putative)	
PRO0019	unknown	
PRO0020	prp protein (putative)	
PRO0029	noduline (putative)	
PRO0058	proteinase inhibitor Rgpi9	seed
PRO0061	beta expansine EXPB9	weak in young flowers
PRO0063	structural protein	young tissues+calli+embryo
PRO0069	xylosidase (putative)	
PRO0075	prolamine 10 Kda	strong in endosperm
PRO0076	allergen RA2	strong in endosperm
PRO0077	prolamine RP7	strong in endosperm
PRO0078	CBP80	
PRO0079	starch branching enzyme I	
PRO0080	Metallothioneine-like ML2	transfer layer of embryo + calli
PRO0081	putative caffeoyl-CoA 3-O-methyltransferase	shoot
PRO0087	prolamine RM9	strong in endosperm
PRO0090	prolamine RP6	strong endosperm
PRO0091	prolamine RP5	strong in endosperm
PRO0092	allergen RA5	
PRO0095	putative methionine aminopeptidase	embryo
PRO0098	ras-related GTP binding protein	
PRO0104	beta expansine EXPB1	
PRO0105	Glycine rich protein	
PRO0108	metallothionein like protein (putative)	
PRO0109	metallothioneine (putative)	
PRO0110	RCc3	strong root
PRO0111	uclacyanin 3-like protein	weak discrimination center / shoot meristem
PRO0116	26S proteasome regulatory particle non-ATPase subunit 11	very weak meristem specific

PRO0117	putative 40S ribosomal protein	weak in endosperm
PRO0122	chlorophyll a/b-binding protein precursor (Cab27)	very weak in shoot
PRO0123	putative protochlorophyllide reductase	strong leaves
PRO0126	metallothionein RiCMT	strong discrimination center / shoot meristem
PRO0129	GOS2	strong constitutive
PRO0131	GOS9	
PRO0133	chitinase Cht-3	very weak meristem specific
PRO0135	alpha-globulin	strong in endosperm
PRO0136	alanine aminotransferase	weak in endosperm
PRO0138	cyclin A2	
PRO0139	Cyclin D2	
PRO0140	Cyclin D3	
PRO0141	cyclophyllin 2	shoot and seed
PRO0146	sucrose synthase SS1 (barley)	medium constitutive
PRO0147	trypsin inhibitor ITR1 (barley)	weak in endosperm
PRO0149	ubiquitine 2 with intron	strong constitutive
PRO0151	WSI18	embryo + stress
PRO0156	HVA22 homologue (putative)	
PRO0157	EL2	
PRO0169	aquaporine	medium constitutive in young plants
PRO0170	High mobility group protein	strong constitutive
PRO0171	reversibly glycosylated protein RGP1	weak constitutive
PRO0173	cytosolic MDH	shoot
PRO0175	RAB21	embryo + stress
PRO0176	CDPK7	
PRO0177	Cdc2-1	very weak in meristem
PRO0197	sucrose synthase 3	
PRO0198	OsVP1	
PRO0200	OSH1	very weak in young plant meristem
PRO0208	putative chlorophyllase	
PRO0210	OsNRT1	
PRO0211	EXP3	
PRO0216	phosphate transporter OjPT1	
PRO0218	oleosin 18kd	aleurone + embryo
PRO0219	ubiquitine 2 without intron	
PRO0220	RFL	

PRO0221	maize UBI delta intron	not detected
PRO0223	glutelin-1	
PRO0224	fragment of prolamin RP6 promoter	
PRO0225	4xABRE	
PRO0226	glutelin OSGLUA3	
PRO0227	BLZ-2_short (barley)	
PRO0228	BLZ-2_long (barley)	

Optionally, one or more terminator sequences may also be used in the construct introduced into a plant. The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. Additional regulatory elements may include transcriptional as well as translational enhancers. Those skilled in the art will be aware of terminator and enhancer sequences, which may be suitable for use in performing the invention. Such sequences would be known or may readily be obtained by a person skilled in the art.

The genetic constructs of the invention may further include an origin of replication sequence which is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

The genetic construct may optionally comprise a selectable marker gene. As used herein, the term "selectable marker gene" includes any gene, which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a nucleic acid construct of the invention. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance, that introduce a new metabolic trait or that allow visual selection. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as nptII encoding neomycin phosphotransferase capable of phosphorylating neomycin and kanamycin, or hpt encoding hygromycin phosphotransferase capable of phosphorylating hygromycin), to herbicides (for example bar which provides resistance to Basta; aroA or gox providing resistance against glyphosate), or genes that provide a metabolic trait (such as manA that allows plants to use mannose as sole carbon source). Visual marker genes result in the formation of colour (for example beta-glucuronidase, GUS), luminescence (such as luciferase) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof). Further examples of suitable selectable marker genes include the ampicillin resistance (Ampr), tetracycline resistance gene (Tcr),

bacterial kanamycin resistance gene (Kanr), phosphinothricin resistance gene, and the chloramphenicol acetyltransferase (CAT) gene, amongst others

The methods of the present invention are particularly relevant for applications in agriculture and horticulture, and serve to develop plants that have altered characteristics.

Accordingly, another embodiment of the invention is a method for making a transgenic plant comprising the introduction of a recombinant nucleic acid as mentioned above into a plant. "A plant" as used herein means plant cell, plant part etc. as defined herein below. According to a preferred embodiment this method for the production of a transgenic plant further comprises the step of cultivating the plant cell under conditions promoting regeneration and mature plant growth.

A further embodiment relates to a method as described above, comprising stably integrating into the genome of a plant a recombinant nucleic acid as mentioned above. Alternatively, the recombinant nucleic acids comprising the nucleic acids of the present invention are transiently introduced into a plant or plant cell. The protein itself and/or the nucleic acid itself may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of the plant). According to a preferred feature of the present invention, the nucleic acid is preferably introduced into a plant by transformation.

The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g. cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively and preferably, the transgene may be stably integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable

ancestor cell. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens *et al.*, 1982; Negrutiu *et al.*, 1987); electroporation of protoplasts (Shillito *et al.*, 1985); microinjection into plant material (Crossway *et al.*, 1986); DNA or RNA-coated particle bombardment (Klein *et al.*, 1987) infection with (non-integrative) viruses and the like.

Transgenic rice plants expressing a gene according to the present invention are preferably produced via *Agrobacterium*-mediated transformation using any of the well known methods for rice transformation, such as described in any of the following: published European patent application EP 1198985 A1, Aldemita and Hodges (1996); Chan *et al.* (1993), Hiei *et al.* (1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida *et al.* (1996) or Frame *et al.* (2002), which disclosures are incorporated by reference herein as if fully set forth.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.

The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

The present invention also encompasses plants obtainable by the methods according to the present invention. The present invention provides plants having one or more altered characteristics, when compared to the wild-type plants, characterised in that the plant has modified expression of one or more nucleic acids and/or modified level and/or activity of a protein, wherein said nucleic acid and/or protein are essentially similar to any one of SEQ ID NO 1 to 2755.

In one embodiment of the present invention, such a plant is a transgenic plant. According to a further embodiment such transgenic plant comprises an isolated nucleic acid and/or protein sequence essentially similar to any one for Seq Id NO 1 to 2755.

Alternatively, according to one embodiment of the present invention, such a plant having one or more altered plant characteristics and having modified expression of one or more nucleic acids and/or modified level and/or activity of a protein, wherein said nucleic acid and/or protein are essentially similar to any one of SEQ ID NO 1 to 2755., is created by breeding techniques.

The present invention clearly extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced in the parent by the methods according to the invention. The invention accordingly also includes host cells containing an isolated nucleic acid molecule encoding a protein essentially similar to any one of SEQ ID NO 1 to 2755. Such host cell may be selected from plants, bacteria, animals, algae, fungi, yeast or insects. Preferred host cells according to the invention are plant cells. The invention also extends to harvestable parts of a plant such as but not limited to seeds, leaves, fruits, flowers, stem cultures, stem, rhizomes, roots, tubers and bulbs.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant" also therefore encompasses suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp., Acer spp., Actinidia spp., Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp.,*

Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chaenomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Diheteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp, Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays, amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, trees. Alternatively algae and other non-Viridiplantae can be used for the methods of the present invention. Preferably the plant according to the present invention is a crop plant selected from rice, maize, wheat, barley, soybean, sunflower, canola, sugarcane, alfalfa, millet, leguminosae (bean, pea), flax , lupinus, rapeseed, tobacco, popular and cotton. Further preferably, the plant according to the present invention is a monocotyledonous plant, most preferably a cereal.

The term 'gene(s)' or 'nucleic acid', 'nucleotide sequence', as used herein refers to a polymeric form of a deoxyribonucleotides or ribonucleotide polymer of any length, either double- or single-stranded, or analogs thereof, that have the essential characteristics of a natural ribonucleotide in that they can hybridize to nucleic acids in a manner similar to naturally occurring polynucleotides. A great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those skilled in the art. For example, methylation, 'caps' and substitution of one or more of the naturally occurring nucleotides with an analog. Said terms also include peptide nucleic acids. The term "polynucleotide" as used herein includes such chemically, enzymatyically or metabolically modified forms of polynucleotides.

With "recombinant nucleic acid" is meant a nucleic acid produced by joining pieces of DNA from different sources through deliberate human manipulation.

The inventors identified genes that are upregulated in plants overexpressing E2Fa/DPa. These genes can be used to simulate E2Fa/DPa related effect in a plant.

Therefore, according to the invention, there is provided a method to alter characteristics of a plant, comprising overexpression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755, or wherein the method comprises enhancing the level and/or activity of one or more proteins essentially similar to a protein sequence essentially similar to any one of SEQ ID NO 1 to 2755.

Also identified were genes that are downregulated in plants overexpressing E2Fa/DPa. These genes can be used to simulate E2Fa/DPa related effect in a plant. Therefore, according to the invention, there is provided a method to alter plant growth characteristics, comprising downregulation of expression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755, or wherein the method comprises decreasing level and/or activity of one or more proteins essentially similar to any one of SEQ ID NO 1 to 2755.

Genetic constructs aimed at silencing gene expression may comprise the nucleotide sequence essentially similar to any one of SEQ ID NO 1 to 2755 or one at least a portion thereof in a sense and/or antisense orientation relative to the promoter sequence. Preferably the portions comprises at least 21 contiguous nucleic acid of a sequence to be downregulated. Also, sense or antisense copies of at least part of the endogenous gene in the form of direct or inverted repeats may be utilized in the methods according to the invention. The characteristics of plants may also be changed by introducing into a plant at least part of an antisense version of the nucleotide sequence essentially similar to any one or more of SEQ ID NO 1 to 2755. It should

be clear that part of the nucleic acid (a portion) could also achieve the desired result. Homologous anti-sense genes are preferred, homologous genes being plant genes, preferably plant genes from the same plant species in which the silencing construct is introduced.

Detailed analysis of the promoters of the genes identified in the present invention allowed the identification of novel E2Fa/DPa target genes that are under the direct control of E2Fa/DPa and that are mainly involved in DNA replication. For all the genes identified in the present invention, reference is made to the MIPS database MATDB accession number. This unique identification number refers to the deposit of information related to the gene in question, e.g. the unspliced sequence, the spliced sequence, the protein sequence, the domains of the protein etc. An example of the information deposited under the accession number At1g57680 is shown in Figure 4. Based on this unique accession number, a person skilled in the art would be able to locate the gene provided by the present invention in its genomic environment. From this information one can identify and isolate the upstream control elements of these genes. Especially interesting are the promoters of these genes as control elements for driving or regulating transcription of heterologous genes. Therefore, according to the invention is provided an isolated nucleic acid comprising one or more of the regulatory elements upstream of the startcodon of the nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755. Furthermore, the invention provides an isolated nucleic acid as mentioned above, wherein said regulatory element is the promoter of said the genes essentially similar to any one of the sequence presented in SEQ ID NO 1 to 2755.

Further the invention also relates to the use of a nucleic acid sequence or protein essentially similar to any one of SEQ ID NO 1 to 2755, for altering plant characteristics.

Another method for altering plant characteristics and/or growth characteristics of a plant resides in the use of allelic variants of the genes of the present invention. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these natural alleles. Alternatively, in particular breeding programs, such as for example marker assisted breeding, or conventional breeding programmes, it is sometimes practical to introduce allelic variation in the plants by mutagenic treatment of a plant. One suitable mutagenic method is EMS mutagenesis. Identification of allelic variants then takes place by, for example, PCR. This is followed by a selection step for selection of superior allelic variants of the sequence in question and which give rise to altered growth characteristics. Selection is typically carried out by monitoring growth performance of plants containing different allelic variants of the sequence in question. Monitoring growth performance can be done in a greenhouse or in the field. Further optional steps include crossing plants in which the superior

allelic variant was identified with another plant. This could be used, for example, to make a combination of interesting phenotypic features.

According to another aspect of the present invention, advantage may be taken of the nucleic acid sequence of the present invention in breeding programs. In such a program, a DNA marker may be identified which is genetically linked to the nucleic acid of the present invention. This DNA marker is then used in breeding programs to select plants having altered growth characteristics. Therefore, the present invention also encompass the use of a nucleic acid sequence essentially similar to any one of SEQ ID NO 1 to 2755, for marker assisted breeding of plants with altered characteristics.

These marker assisted breeding processes may further involve the steps of crossing plants and using probes or primers having part, for example having at least 10 bp, of a sequence corresponding to any of SEQ ID NO 1 to 2755, to detect the DNA sequence corresponding to SEQ ID NO 1 to 2755, in the progeny of the cross.

These methods for marker assisted breeding also may involve the use of an isolated DNA molecule being essentially similar to SEQ ID NO 1 to 2755 or a part thereof as a marker in techniques like AFLP, RFLP, RAPD, or in the detection of Single Nucleotide Polymorphisms.

Further these methods for marker assisted breeding also may involve determining the presence or absence in a plant genome of a qualitative trait or a quantitative trait locus (QTL) linked to a transgene essentially similar to any one of SEQ ID NO 1 to 2755 or to an endogenous homologue of any one of SEQ ID NO 1 to 2755, which method comprises:

(a) detecting a molecular marker linked to a QTL, wherein the molecular marker comprises a sequence essentially similar to SEQ ID NO 1 to 2755or an endogenous homologue thereof; and

(b) determining the presence of said QTL as by detection of the molecular marker of step (a) or determining the absence of said QTL as failure to detect the molecular marker of step (a)

Alternatively, methods for marker assisted breeding may involve detecting the presence of a quantitative trait locus linked to a DNA sequence essentially similar to SEQ ID NO 1 to 2755 or to an endogenous homologue thereof in the genome of a plant. The methods described above may involve the steps of:

(a) extracting a DNA sample of said plant;

(b) contacting the DNA sample with a probe that hybridises to a DNA sequence according to claim 1 or to an endogenous homologue thereof, or to the complement thereof;

(c) performing a hybridisation reaction under conditions suitable for hybridisation of the probe to the DNA sample of (b); and

(d) detecting the hybridisation of the probe to the DNA.

Further, the present invention also encompass the use of a nucleic acid sequence essentially similar to any one of SEQ ID NO 1 to 2755, for conventional breeding of plants with altered characteristics.

In conventional breeding programs, the nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755 is used to select plants with better plant characteristics compared to the normal wild-type plants. The plants with better growth characteristics may originated from natural variation in the alleles of the gene corresponding to any one of SEQ ID NO 1 to 2755, or may originated from manmade variation in these genes, for example variation created by EMS mutagenesis or other methods to created single nucleotide polymorfisms.

Further the invention also relates to the use of a nucleic acid or a protein essentially similar any one of SEQ ID NO 1 to 2755, as a growth regulator.

In a particular embodiment such a growth regulator is a herbicide or is a growth stimulator. The present invention therefore also provides a growth regulating composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755. The growth regulating compositions according to the present invention can additionally comprise any additive usually present in growth regulating compositions such as growth inhibitors, herbicides or growth stimulators. Also a kit comprising a sequence essentially similar to any one of SEQ ID NO 1 to 2755 (for example in the form of a herbicide) is in the scope of the present invention. Also any other plant effective agent comprising the sequences according to the present invention are provided herein. Methods to produce the compositions, kits or plant agents as mentioned above are also provided by the present invention and involve the production of any one or more of the sequences essentially similar to any one of SEQ ID NO 1 to 2755. Such sequences and methods are herein provided.

Further, plants of the present invention have improved characteristics, such as improved growth and yield, which makes these plant suitable to produce industrial proteins.

Accordingly, the present invention provides a method for the production of enzymes and/or pharmaceuticals, which method comprises modifying expression of a nucleic acid, and/or modifying level and/or activity of a protein, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755

The present invention therefore also encompasses the use of plants as described above, for the production of (industrial) enzymes and/or pharmaceuticals. The (Industrial) enzymes and pharmaceuticals produced according to the method as described above are also encompasses by the present invention.

Also the invention as presented herein offers means to alter the characteristics not only of plants, but also of other organisms, such as mammals. The plant genes of the present invention, or their homologues, or the plant proteins or their homologues, can be used as therapeutics or can be used to develop therapeutics for both humans and animals. Accordingly, the present invention relates to a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755 , for use as a therapeutic agent.

In a particular embodiment, the use as a therapeutic agents encompasses the use in gene therapy, or the use to manufacture medicaments such as for example therapeutic protein samples. Also the nucleic acids and/or proteins according to the present invention can be applied in diagnostic methods.

Accordingly provided by the present invention is the use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755, for use as a therapeutic agent, a diagnostic means, a kit or plant effective agent.

Further encompassed by the invention are therapeutic or diagnostic compositions or kits or plant effective agent, comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755. These compositions may comprise other additives usually applied for therapeutic compositions. Methods to produce these therapeutic or diagnostic compositions or kts are also provided by the present invention and involve the production of any of the sequences essentially similar to any one of SEQ ID NO 1 to 2755.

The plants according to the present invention have altered characteristics, such as for example improved growth and yield, which makes them suitable sources for many agricultural applications and the food industry. Accordingly, provided by the present invention there is a food product derived from a plant or host cell as described above and also the use of such a food product in animal feed or food.

In molecular biology it is standard practice to select upon transfection or transformation those individuals (or groups of individuals, such as bacterial or yeast colonies or phage plaques or eukaryotic cell clones) that are effectively transfected or transformed with the desired genetic

construct. Typically these selection procedures are based on the presence of a selectable or screenable marker in the transfected genetic construct, to distinguish the positive individuals easily from the negative individuals. The nucleic acids and proteins according to the present invention are capable of altering the characteristics of the host cells to which they are applied. Therefore, the nucleic acids and/or proteins according to the present invention can also be used as selectable markers, screenable markers or selection agents. According to one particular embodiment, the present invention provides the use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755 as a positive or negative selectable marker during transformation of plant cell, plant tissue or plant procedures.

DESCRIPTION OF THE FIGURES

Figure 1: Volcano plot of significance against effect. Each x represent one of the 4579 genes, with the negative log10 of the P value from the gene model plotted against the difference between least-square means for the genotype effect. The horizontal line represents the test-wise threshold of P=0.05. The two vertical reference lines indicate a 2-fold cutoff for either repression or induction.

Figure 2: Sources of alpha- ketoglutarate and other metabolites in plants, with annotation of up and downregulated genes in the E2Fa-DPa overproducing cells. Upregulated enzymes are underlined with a dashed line and enzymes underlined with a full line are downregulated in the E2Fa-DPa versus wild type plants. Products that are boxed act as precursors for nucleotide biosynthesis. A -KG, alpha-ketoglutarate; GOGAT, glutamate synthetase; NIA2, nitrate reductase, NiR, nitrite reductase.

Figure 3: Endoreduplication levels in wild type and E2Fa/DPa transgenic lines in relation to nitrogen availability. Wild type (A) and transgenic (B) lines were grown on minimal medium in the presence of 0.1, 1, 10, or 50 mM ammonium nitrate. Values are means of three independent measurements.

Figure 4: Represents the information which is deposited in the MatDB (MIPS *Arabidopsis* database) under accession number At1g57680

Figure 5: Verification of microarray analysis by RT-PCR. RT-PCR analysis was carried out under linear amplification conditions. The actin 2 gene (ACT2) was used as loading control. GS, glutamine synthetase; GOGAT, glutamate synthase; NiR, nitrite reductase.

Figure 6: NMR spectrum of E2Fa/DPa overexpressing plant cells.

Table 1: Presentation of *Arabidopsis* genes that are 2 fold or more upregulated in E2Fa-DPa overexpressing plants. The genes are presented according to the functional category to which they belong. For some of the genes, no function has been described in the public databases and they are named unknown, putative or hypothetical protein. All the genes have each a unique MIPS accession number, which refers to the identification of the sequence in the MatDB (MIPS *Arabidopsis thaliana* database). The MIPS accession number refers to the protein entry code for the MatDB of MIPS. Also, there is an accession number provided as an internal protein code. The fold of induction is also given for each sequence. Furthermore, where an E2F target sequence has been identified in the upstream region of the gene, the sequence of that site is also presented in the Table. Finally, other plant homologues which have substantial sequence identity with the *Arabidopsis* gene are mentioned in this Table.

Table 2: Presentation of *Arabidopsis* genes that are 2 fold or more repressed in E2Fa-DPa overexpressing plants. Data are presented in a similar way as for Table 1, as explained above.

Table 3: Different E2F target sequences and the frequency of their presence in the upstream regions of the *Arabidopsis* genes described in the present invention.

Table 4: Selection of the *Arabidopsis* genes from the microarray that were 1.3 times upregulated in E2Fa/DPa overexpressing plants, compared to the wild–type plants. The gene name is given, as well as the MIPS database accession number and a ratio indicating the degree of upregulation of the gene. Furthermore, the E-value indicates if a significant homologue has been found in the public databases.

Table 5: Selection of the *Arabidopsis* genes from the microarray that were 1.3 times repressed in E2Fa/DPa overexpressing plants, compared to the wild –type plants. The data are presented as in Table 4. The fold repression is calculated as 1/ratio. In this Table only the genes that have a ratio of less than 0.77 are selected.

Table 6: genes selected for Arabidopsis transformation

Table 7: genes selected for rice transformation

EXAMPLES

Example 1. Overexpression of E2Fa and DPa in Arabidopsis

Double transgenic CaMV35S-E2Fa-DPa overexpressing plants were obtained by the crossing of homozygous CaMV35S-E2Fa and CaMV35S-DPa plants (De Veylder et al., 2002). Double transformants were grown under a 16h light/ 8h dark photoperiod at 22°C on germination medium (Valvekens et al., 1988).

Selection of transgenic lines

Arabidopsis thaliana plants were generated that contained either the *E2Fa* or the *DPa* gene under the control of the constitutive cauliflower mosaic virus (*CaMV*) *35S* promoter.

Crossing experiments of overexpressing E2Fa and DPa lines

Plants homozygous for the *CaMV 35S E2Fa* gene were crossed with heterozygous *CaMV 35S DPa* lines. Polymerase chain reaction (PCR) analysis on individual plants confirmed which plants contained both the *CaMV 35S-E2Fa* and *CaMV 35S-DPa* constructs.

8 days after sowing, these plants were used to isolate total RNA, from which cDNA was synthesized and subsequently hybridized to a microarray containing 4579 unique *Arabidopsis* ESTs. These experimental steps are described in the following examples.

Example 2: Construction of Microarrays

Construction of Microarrays

The *Arabidopsis* thaliana microarray consisted of 4,608 cDNA fragments spotted in duplicate, distant from each other, on Type V silane coated slides (Amersham BioSciences, Buckinghamshire, UK). The clone set included 4,579 *Arabidopsis* genes composed from the unigen clone collection from Incyte (*Arabidopsis* Gem I, Incyte, USA). To retrieve the functional annotation of the genes relating to the spotted ESTs, BLASTN against genomic sequences was performed. To make the analysis easier a collection of genomic sequences bearing only one gene was built according to the available annotations. Each of those sequences had its upstream intergenic sequence followed by the exon-intron structure of the gene and the downstream intergenic sequence, intergenic being the whole genomic sequence between start and stop codons from neighboring protein-encoding genes. From the BLASTN output the best hits were extracted and submitted to a BLASTX search against protein databases. To retrieve even more detailed information concerning the potential function of the genes, protein domains were searched using ProDom. The complete data set can be found on the website http://www.psb.rug.ac.be/E2F and is cited herein by reference. The cDNA inserts were PCR amplified using M13 primers, purified with MultiScreen-PCR plate (cat: MANU03050, Millipore,

Belgium) and arrayed on the slides using a Molecular Dynamics Generation III printer (Amersham BioSciences). Slides were blocked in 3.5%SSC, 0.2%SDS, 1% BSA for 10 minutes at 60°C.

5

RNA amplification and labeling

Antisense RNA amplification was performed using a modified protocol of *in vitro* transcription as described earlier in Puskas et al. (2002). For the first strand cDNA synthesis, 5 µg of total RNA was mixed with 2 µg of an HPLC-purified anchored oligo-dT + T7 promoter (5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-T_{24}(A/C/G)-3`) (SEQ ID NO 2756). (Eurogentec, Belgium), 40 units of RNAseOUT (cat# 10777-019, Invitrogen, Merelbeke, Belgium) and 0.9M D(+) trehalose (cat# T-5251, Sigma Belgium) in a total volume of 11µl, and heated to 75°C for 5 minutes. To this mixture, 4 µl 5x first strand buffer (Invitrogen, Belgium), 2 l 0.1 M DTT, 1 µl 10 mM dNTP mix, 1 µl 1.7 M D(+)trehalose (cat# T-5251, Sigma Belgium) and 1 µl, 200 Units of SuperScript II (cat#: 18064-014, Invitrogen, Belgium) was added in 20 µl final volume. The sample was incubated in a Biometra-UnoII thermocycler at 37°C for 5 minutes, 45°C for 10 minutes, 10 cycles at 60°C for 2 minutes and at 55°C for 2 minutes. To the first strand reaction mix, 103.8 µl water, 33.4 µl 5x second strand synthesis buffer (Invitrogen, Belgium), 3.4 µl 10 mM dNTP mix, 1 µl of 10U/µl E.coli DNA ligase (cat#: 18052-019, Invitrogen, Belgium), 4 µl 10 U/µl E.coli DNA Polymerase I (cat#: 18010-025, Invitrogen, Belgium) and 1 µl 2U/µl E.coli RNAse H (cat#: 18021-071, Invitrogen, Belgium) was added, and incubated at 16°C for 2 hours. The synthesized double-stranded cDNA was purified with Qiaquick (cat#: 28106, Qiagen, Hilden, Germany). Antisense RNA synthesis was done by AmpliScribe T7 high yield transcription kit (cat#: AS2607; Epicentre Technologies, USA) in total volume of 20 µl according to the manufacturer's instructions. The RNA was purified with RNeasy purification kit (cat#: 74106; Qiagen, Germany). From this aRNA, 5 µg was labeled by reverse transcription using random nonamer primers (Genset, Paris, France), 0.1 mM d(G/T/A)TPs, 0.05 mM dCTP (Amersham BioSciences, UK), 0.05 mM Cy3-dCTP or Cy5-dCTP (cat#: PA53023, PA55023; Amersham BioSciences, UK) 1x first strand buffer, 10 mM DTT and 200 Units of SuperScript II (cat#: 18064-014, Invitrogen, Belgium) in 20 µl total volume. The RNA and primers were denatured at 75°C for 5 minutes and cooled on ice before adding the remaining reaction components. After 2 hours incubation at 42°C, mRNA was hydrolyzed in 250 mM NaOH for 15 minutes at 37°C. The sample was neutralized with 10 µl of 2 M MOPS and purified with Qiaquick (cat#: 28106, Qiagen, Germany).

Array hybridization and post-hybridization processes

The probes were resuspended in 30 µl hybridization solution (50 % formamide, 5x SSC, 0.1 % SDS, 100 mg/ml salmon sperm DNA) and prehybridized with 1µl poly-dT (1mg/ml) at 42°C for 30 minutes to block hybridization on the polyA/T tails of the cDNA on the arrays. 1 mg/ml mouse COT DNA (cat#: 18440-016, Invitrogen, Belgium) was added to the mixture and placed on the array under a glass coverslip. Slides were incubated for 18 hours at 42°C in a humid hybridization cabinet (cat#: RPK0176; Amersham BioSciences, UK). Post-hybridization

washing was performed for 10 minutes at 56°C in 1xSSC, 0.1% SDS, two times for 10 minutes at 56°C in 0.1xSSC, 0.1% SDS and for 2 minutes at 37°C in 0.1xSSC.

Scanning and data analysis

Arrays were scanned at 532 nm and 635 nm using a Generation III scanner (Amersham BioSciences, UK). Image analysis was performed with ArrayVision (Imaging Research Inc, Ontario, Canada). Spot intensities were measured as artifact removed total intensities (ARVol). No background correction was performed. First, within-slide normalization was addressed by plotting for each single slide a "MA-plot" (Yang et al., 2002), where $M = \log_2 (R/G)$ and $A = \log_2 0.5\sqrt{R \times G}$. The "LOWESS" normalization was applied to correct for dye-intensity differences. Subsequently, in order to normalize between slides and to identify differentially expressed genes between the two genotypes, two sequential analyses of variance (ANOVAs) were applied, proposed by Wolfinger et al. (2002), as follows: 1) firstly, the base-2 logarithm of the "LOWESS"-transformed measurements for all 73,264 spots (y_{gklm}) was subjected to a normalization model of the form $y_{iklm} = \mu + A_k + A_k D_l R_m + \varepsilon_{iklm}$, where μ is the sample mean, A_k is the effect of the kth array (k = 1-4), $A_k D_l R_m$ is the channel-effect (AD) for the mth replication of the total collection of cDNA fragments (m = 2; left or right), and ε_{iklm} is the stochastic error; 2) secondly, the residuals from this model were subjected to 4,579 gene-specific models of the form $r_{ijkl} = \mu + G_i A_k + G_i D_l + G_i C_j + \gamma_{ijkl}$ where $G_i A_k$ is the spot effect, $G_i D_l$ is the gene-specific dye effect, $G_i C_j$ is representing the signal intensity for genes that can specifically be attributed to the genotypes (effect of interest), and γ_{ijkl} is the stochastic error. All effects were assumed to be fixed effects, except ε_{klm} and γ_{ijkl}. A t-test for differences between the $G_i C_j$ effects was performed, where the t-tests are all based on n_1+n_2-2 degrees of freedom corresponding to the n_1 WT hybridisations and n_2 E2Fa-DPa hybridisations. The p-value cutoff was set at 0.01. No further adjustment for multiple testing was performed, as Bonferroni adjustment for 4,579 tests, to assure an experiment-wise false positive rate of 0.05, results in a p-value cutoff of $1e^{-5.0}$, which is certainly too conservative; therefore it was chosen to set the p-value cutoff arbitrarily at the 0.01 level. Also $G_i D_l$ effects were estimated and t-tested for significance at the 1% level in a same way as described above. Genes with a significant $G_i D_l$ effect were discarded. Genstat was used to perform both the normalization and gene model fits.

Example 3: Results of the Microarray analysis and statistical analysis

A micro-array containing in duplicate 4579 unique *Arabidopsis* ESTs, representing about a sixth of the total genome, was used to compare the transcriptome of wild type with that of *E2Fa-DPa* overexpressing plants. cDNA was synthesized from total RNA isolated from wild type and transgenic plants harvested 8 days after sowing. At this stage, transgenic plants were distinguished from control plants by the appearance of curled cotelydons which display ectopic

cell divisions and enhanced endoreduplication (De Veylder et al., 2002). In the first two hybridizations Cy3 and Cy5 fluorescently labeled probe pairs of control and *E2Fa-DPa* cDNA were used using independent mRNA extractions of the *E2Fa-DPa* plants. Subsequently, a dye-swap replication was performed for both hybridizations, resulting in a total of four cDNA microarray hybridizations.

Fluorescence levels were analyzed with the aim to establish whether the level of expression of each gene varied according to overexpression of the *E2Fa-DPa* transcription factor. Two sequential analyses of variance (ANOVAs) were used, as proposed by Wolfinger et al. (2002). The first ANOVA model, called the "normalization" model, accounts for experiment-wise systematic effects, such as array- and channel-effects, that could bias inferences made on the data from the individual genes. The residuals from this model represent normalized values and are the input data for the second ANOVA model, called the "gene" model. The gene models are fit separately to the normalized data from each gene. This procedure uses differences in normalized expression levels, rather than ratios, as the unit of analysis of expression differences.

Prior to the estimation of genotype-specific signal intensities of the genes (G_iC_j effects), which are the effects of interest, gene-specific dye effects (G_iD_l effects) were estimated and t-tested for significance at the 1% level. One hundred and thirty one genes showed a significant G_iD_l effect and were discard from further analysis. For each of the remaining 4,448 genes on the arrays, a t-test on the G_iC_j effects for significant differences ($p<0.05$) was performed. Figure 1 plots the obtained p-values (as the negative log10 of the p-value) against the magnitude of the effect (log2 of estimated fold change). This volcano plot illustrates the substantial difference significance testing can make versus cutoffs made strictly on the basis of the fold change. The two vertical reference lines indicate a 2-fold cutoff for either repression or induction, while the horizontal reference line refers to the p-value cutoff at the 0.05 value. These reference lines divide the plot into six sectors. The 3,535 genes in the lower middle sector have low significance and low fold change, and both methods agree that the corresponding changes are not significant. The 188 genes in the upper left and right sectors have high significance ($p<0.05$) and high fold change (≥ 2); 84 of these genes show a significant two-or-more-fold induction of expression, where the remaining 104 genes show a significant two-or-more-fold repression of expression in the E2Fa-DPa plant. Finally, the 715 genes in the upper middle sector represent significant ($p<0.05$) up- or downregulated genes, but with a low (≤ 2) fold change. The full dataset of genes can be viewed at http://www.psb.rug.ac.be/E2F, which dataset is incorporated herein by reference.

All the sequences that are 1.3 times upregulated (ratio of more than 1.999) in E2Fa-DPa overexpressing plants are presented in Table 4. All the sequences that are 1.3 times repressed (calculated as 1/ ratio of less than 0.775) are presented in Table 5. Particularly interesting genes that are more than 2-fold upregulated or 2 fold repressed are selected and separately represented in Tables 1 and 2.

Example 4: sequencing and RT mediated PCR analysis

The identity of the genes was confirmed by sequencing, and the induction of a random set of genes was confirmed by RT-PCR analysis (Figure 5).

RNA was isolated from plants 8 days after sowing with the Trizol reagent (Amersham Biosciences). First-strand cDNA was prepared from 3 µg of total RNA with the Superscript RT II kit (Invitrogen) and oligo(dT)18 according to the manufacturer's instructions. A 0.25 µl aliquot of the total RT reaction volume (20 µl) was used as a template in a semi-quantitative RT-mediated PCR amplification, ensuring that the amount of amplified product remained in linear proportion to the initial template present in the reaction. From the PCR reaction, 10 µl was separated on a 0.8% agarose gel and transferred onto Hybond N+ membranes (Amersham Biosciences) that were hybridized at 65°C with fluorescein-labeled probes (Gene Images random prime module; Amersham Biosciences). The hybridized bands were detected with the CDP Star detection module (Amersham Biosciences). Primers used were 5'-AAAAAGCAGGCTGTGTCGTACGATCTTCTCCCGG-3' (SEQ ID NO 2757) and 5'-AGAAA GCTGGGTCATGTGATAGGAGAACCAGCG-3' (SEQ ID NO 2758) for E2Fa, 5'-ATAGAA TTCGCTTACATTTTGAAACTGATG-3' (SEQ ID NO 2759) and 5'-ATAGTCGACTCAGCGA GTATCAATGGATCC-3' (SEQ ID NO 2760) for DPa, 5'-CAGATCTTGTTAACCTTGACAT CTCAG-3' (SEQ ID NO 2761) and 5'-GGGTCAAAAGATACAACCACACCAG-3' (SEQ ID NO 2762) for glutamine synthetase (GS), 5'-GGTTTACGAGCTACATGGCCC-3' (SEQ ID NO 2763) and 5'-GAGCAATCCGTTCAGCCTCC-3' (SEQ ID NO 2764) for glutamate synthase (GOGAT), 5'-GCGTTTGACCACTCTTGGAGAC-3' (SEQ ID NO 2765) and 5'-GAACGCCA TTGAGAAAGTCCGC-3' (SEQ ID NO 2766) for histone acetylase HAT B, 5'-GTTACCGG CTCGACTTGAAGATC-3' (SEQ ID NO 2767) and 5'-GAATCGGAGGGAAAGTCTGACG-3' (SEQ ID NO 2768) for LOB domain protein 41, 5'-GTGTGGTTTCCAAGCTTTCCTACG-3' (SEQ ID NO 2769) and 5'-GGTGAAGGGACTAGCCTTGTGG-3' (SEQ ID NO 2770) for isocitrate lyase, 5'-GGGATCAATCCTCAGGAGAAGG-3' (SEQ ID NO 2771) and 5'-CCGTCCATCTTTATTAGCGGCATG-3' (SEQ ID NO 2772) for nitrite reductase (NiR), and 5'-TTACCGAGGCTCCTCTTAACCC-3' (SEQ ID NO 2773) and 5'-ACCACCGATCCAGACA CTGTAC-3' (SEQ ID NO 2774) for actin 2 (ACT2).

Example 5: Characterization of the genes identified as being involved in E2F/DP regulated cellular processes

The genes of the present invention identified from the microarray experiment of Example 2 have unique identification numbers (MIPS accession number e.g. At1g57680). The MIPS accession number is widely accepted in this field as it directly refers to the genomic sequence and the location of the sequence in the Arabidopsis thaliana genome. Accession numbers are allocated by the Munich Information Center for Protein Sequences (MIPS) and are stored in the MIPS *Arabidopsis* database. Publicly available sequence and annotation data from all other AGI ("*Arabidopsis* Genome Initiative") groups are included to establish a plant genome database (Schoof H, et al. (2002)). The MIPS *Arabidopsis* database can be accessed via the Internet http://mips.gsf.de/cgi-bin/proj/thal and the database can be searched with the protein entry code (e.g. At1g57680). An example of the type of sequence information and protein domain information that is provided for a certain sequence in the MIPS database is shown Figure 4.

An additional blast search with the genes according to the present invention was performed on public databases containing sequences from other plant species and other organisms. For some of the genes identified by the microarray, significant levels of homology (low E-values) were found with sequences from other organisms (see Tables 1 and 2 with reference to their Genbank accession number). So far, mainly corn and rice homologues were identified, but as more genomes will be sequenced in the future, many more homologues will be identifiable by the person skilled in the art as useful in the methods of the present invention.

DNA replication and cell cycle genes

Genes up or downregulated in the *E2Fa-DPa* overexpressing plants can be classified into clear groups according their function (Tables 1 and 2). 14 Genes that are 2-fold or more upregulated belong to the class of genes involved in DNA replication and modification, correlating with the observation that E2Fa-DPa overexpression plants undergo extensive endoreduplication. Most of these genes have previously be shown to be upregulated by *E2F-DP* overexpression in mammalian systems including a putative thymidine kinase, replication factor c, and histone genes (4 different ones). Other E2Fa-DPa induced S phase genes include a linker histone protein, the topoisomerase 6 subunit A and two subunits of the histone acetyltransferase HAT B complex, being HAT B and Msi3. The HAT B complex is responsible for the specific diacethylation of newly synthesized histone H4 during nuclease assembly on newly synthesized DNA (Lusser et al., 1999). Also a DNA methyltransferase responsible for the methylation of cytosine in cells that progress though S phase was identified among upregulated genes.

Besides the overexpressed E2Fa gene (being 90-fold more abundant in the E2FaPa overexpressing plants, compared to control plants), only one cell cycle gene (*CDKB1;1*) shows a 2-fold or more change in expression level upon *E2Fa-DPa* overexpression. CDKB1;1 was previously predicted to be a candidate E2F-DP target by virtue of a consensus E2F-DP-binding site in its promoter (de Jager et al., 2001). Whereas CDKB1;1 activity is maximum at the G2/M transition, its transcript levels start to rise during late S-phase (Porceddu et al., 2001; Menges and Murray, 2002). Upregulation of *CDKB1;1* might therefore be a mechanism to link DNA replication with mitosis.

Cell wall biogenesis genes

Four members of the xyloglucan endotransglucosylase (XET) gene family were found to be 2-fold or more upregulated in E2Fa-DPa overexpressing plants, one of them identical to the Meri-5 gene (Medford et al., 1991). XETs are enzymes that modify cell wall components and therefore play a likely role in altering size, shape and physical properties of plant cells. Reversal breakage of the xyloglucan tethers by XETs has been proposed to be a mechanism for allowing cell wall loosening in turgor-driven cell expansion (Campbell and Braam, 1999). However, there are several reasons to believe that E2Fa-DPa induced XETs are not required for cell expansion. First, cells divide more frequently in E2Fa-DPa overexpressing plants, but the overall cell size of the cells is smaller. Therefore, no overall increase in expansion-rates is needed. Second, correlated with the absence of increased cell expansion in the transgenic lines, no induction of genes with a known role in this process, such as expansins, can be seen. Therefore, the hydrolytic activity of the XETs might be required to incorporate the newly synthesized cell walls formed during cytokinesis into the existing cell wall structure. Alternatively, as XET activity has shown to be involved in the postgerminative mobilization of xyloglucan storage reverses in Nasturtium cotelydons (Farkas et al., 1992; Fanutti et al., 1993), induction of XETs in E2Fa-DPa overexpressing plants might relate to polysaccharide breakdown to serve the metabolic and energy needs which are required to synthesize new nucleotides (see below).

Interestingly, two XETs were identified in the set of 2-fold or more downregulated genes. These XETs are more related to each other than to the induced XET proteins. This differential response of XETs towards the E2Fa/DPa induced phenotypes suggests that plant XETs can be classified in at least 2 different functional classes.

Genes involved in metabolism and biogenesis

Both the group of up and down regulated genes contains a relative large group of genes involved in metabolism and biogenesis. Most remarkable is the induction of genes involved in nitrogen assimilation, such as nitrate reductase (NIA2) (see Figure 2), glutamine synthetase (GS), and glutamate synthetase (GOGAT). Although not present on the microarray, the nitrite reductase (NiR) gene was found to be induced in the transgenic line, as demonstrated by RT-mediated PCR analysis. Nitrogen and nitrite reductase catalyse the first step in the nitrogen assimilation pathway, whereas glutamine and glutamate synthetase are involved in both the primary assimilation from nitrogen as reassimilation of free ammonium, supplying all plants nitrogen needed for the biosynthesis of amino-acids and other nitrogen-containing compounds.

There are other indications that nitrogen metabolism is altered in E2Fa-DPa overexpressing plants, such as the modification of genes reported to be involved in Medicago induced nodulation (MTN3 and a nodulin-like gene); and the downregulation of genes involved in sulfur assimilation (adenylylsulfate reductase (APR; 2 different genes) and a putative adenine phosphosulfate kinase). Genes involved in sulfur assimilation such as APR have previously been shown to be transcriptionally downregulated during nitrogen deficiency (Koprivova et al., 2000).

Upregulation of nitrogen assimilation genes in E2Fa-DPa overexpressing plants might reflect the need for nitrogen for nucleotide biosynthesis, as purine and pyrimidine bases are nitrogen-rich. If nitrogen assimilation was indeed stimulated by E2Fa/DPa overexpression, two requirements should be fulfilled. Since nitrogen assimilation through the GS/GOGAT pathway requires α-ketogluterate (Lancien et al., 2000), a first requirement is that there should be enough α-ketogluterate to act as acceptor molecule for ammonium. Secondly, because assimilation of nitrogen is energy consuming, the rate of reductant production should be higher in an E2Fa/DPa transgenic than in wild-type plants.

Our micro-array data suggest that in the *E2Fa-DPa* overexpressing plants, α-ketogluterate accumulation is stimulated in different ways. First, α-ketogluterate production is improved by increased photosynthetic activity, as indicated by the 4.7-fold upregulation of large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Figure 2). This results in an accumulation of glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate can be converted into fructuse-1,6-bisphosphate by fructose bisphosphate aldolase. However, a 6-fold downregulation of the fructose bisphosphate aldolase gene rather suggests the conversion of glyceraldehyde-3-phosphate into pyruvate, which can be converted into α-ketogluterate during glycolysis in the citrate cycle. The preferential conversion of glyceraldehyde-3-phosphate into pyruvate in favour of sugars fits the higher need for amino-acids than for sugars for nucleotide

biosynthesis. A shortage for ribose-5-phosphate for nucleotide synthesis is also evident from a downregulation of sucrose-phosphate synthase, resulting in decreased conversion of fructose-6-phosphate and glucose-6-phosphate into sucrose (Figure 2).

A second source of α-ketogluterate is provided in the glyoxylate cycle by the 3.1 fold increase in expression of isocitrate lyase, suggesting an increased lipid turnover in E2Fa-DPa overexpressing plants. Isocistrate lyase activity cleaves isocitrate into glyoxylate and succinate (Figure 2). Whereas the formed glyoxylate can be converted into glycine, which is also required for nucleotide biosynthesis, succinate can be converted into α-ketogluterate in the citrate cycle. A 2.3-fold decrease of the fumarase gene presumably stimulates the conversion of produced α-ketogluterate into glutamate by causing an accumulation of succinate and fumarate, which is also a side product formed during nucleotide biosynthesis (Figure 2).

Assimilation of nitrogen is energy consuming. When rates of nitrate reduction are high, this pathway becomes the major sink for reductant. About 10% of the electron flux in photosynthesizing leaves is used for nitrate reduction. The amount of required reductant, which in leaves originates from electronic photosynthetic electron transport, is therefore expected to be higher in the E2Fa-DPa transgenics. Correspondingly, several components of the chloroplast electron transport chain and associated ATP-synthesing apparatus, such as cytochrome B6, a PSII subunit and the ATPase epsilon subunit are upregulated in the transgenic plants. Increased expression of the protochlorophyllide reductase precursor suggests that an increase in chlorophyll biosynthesis is stimulated in E2Fa-DPa overexpressing plants.

Famine of nitrogen has a putative impact on amino-acid biosynthesis, as three different amino-acid aminotransferases, are downregulated in E2Fa-DPa overexpressing plants. Accompanied with a putative decreased aminotransferase activity is the observed reduction in expression of an enzyme involved in pyridoxine biosynthesis. Shortage of nitrogen-rich amino-acids is also evident from reduced expression of genes encoding vegetative storage proteins (VSP1and VSP2); and ERD10, a protein with a compositional bias towards glu (Kiyosue et al., 1994). Additional evidence for amino acid shortage comes from downregulation of a myrosinase-binding protein and cytochrome P450 monooxygenase CYP83A1. Both proteins are involved in the biosynthesis of glucosinolates, being nitrogen and sulfur containing products derived from amino-acids (Wittstock and Halkier, 2002).

Transcription factors and signal transduction

A total of 4 transcription factors were identified among the genes being 2-fold or more upregulated, including two homeobox domain transcription factors. Among them the anthocyaninless2 (ANL2) gene was identified, which is involved in anthocyanin accumulation in subepidermal leaf cells (Kubo et al., 1999). The lack of an obvious increase in anthocyanin accumulation in E2Fa-DPa overexpressing plants suggests a role for the ANL2 protein in plant development different from anthocyanin production. This hypothesis is substantiated by the observation that anl2 mutant plants contain extra cells in the root between the cortical and epidermal layers (Kubo et al., 1999).

The second upregulated homeobox domain transcription factor is Atbh-6. Expression of Atbh-6 is restricted to regions of cell division and/or differentiation and has been shown to be inducible by water stress and ABA (Soderman et al., 1999). Other putative ABA sensitive genes can be recognized among the E2Fa-DPa induced clones, as well as the cold regulated protein COR6.6, a seed imbitition-like protein and a dormancy-associated protein. Here again, changes in expression level of these genes might be correlated with modifications in carbon metabolism. A link between ABA and sugar signaling is evident from the identification of several loci involved in both sugar and hormonal responses (Finkelstein and Gibson, 2002). Alternatively, it might be the occurrence of enhanced endoreduplication and/or cell division itself that causes a change in the osmotic potential.

Among the downregulated transcription factors a DOF family member is present. Many DOF transcription factors are participating in the regulation of storage protein genes and genes involved in carbon metabolism (Gualberti et al., 2002). Its downregulation might therefore be linked with the shortage of amino-acids due to the high demand of nitrogen for nucleotide biosynthesis.

Other regulatory genes modified in E2Fa-DPa overexpressing plants include protein kinases, several putative receptor kinases, a putative phytochrome A, and WD-40 repeat containing proteins (Tables 1 and 2). Interestingly, a SNF1-like kinase is downregulated 2-fold in E2Fa-DPa overexpressing plants. In addition to its proposed role in sugar signaling, the SNF1 kinase also negatively regulates the activity of plant nitrate reductase (Smeekens, 2000).

Example 6: Endoreduplication levels of E2Fa-DPa plants are nitrogen dependent

The modified expression of a large number of metabolic and regulatory genes, directly or indirectly linked to nitrogen metabolism, suggests a direct relationship between the high endoreduplication levels found in the E2Fa/DPa transgenic plants and nitrogen availability. To test this hypothesis, wild type and transgenic plants were grown on medium containing

different levels of ammoniumnitrate, ranging from 0.1 to 50 mM. Eight days after germination ploidy levels in these plants was determined by flow cytometry. Increasing ammoniumnitrate levels hardly had an effect on the ploidy distribution pattern in wild type plants (Figure 3A). In contrast, in the E2Fa-DPa transgenic plants increasing ammoniumnitrate levels resulted in a reproducible and significant increase in the amount of 32C and 64C nuclei (Figure 3B). Comparing the lowest with the highest concentration of ammonimumnitrate, an increase of 32C from 2.0 (± 0.3) % to 6.5 (± 1.5) %, and of 64C from 0.7 (±0.3) % to 2 (± 0.5) % can be seen. Increasing ammonium levels did not have any effect on the plant phenotype, as plants remained small with curled leaves on all concentrations of nitrogen tested. These data indicate that the endoreduplication levels in the E2Fa-DPa overexpressing plants are limited by nitrogen availability, and that an excess of nitrogen is incorporated into new DNA than in other nitrogen containing compounds.

Example 7: Promoter analysis of E2Fa-DPa regulated genes

Promoter analysis

The intergenic sequence corresponding to the promoter area of each gene spotted on the microarray was extracted from genomic sequences. These genomic sequences are easily accessible for example from the MIPS MatDB database (http://mips.gsf.de/proj/thal/db/). From those intergenic sequences, up to 500bp upstream of the ATG start codon were extracted and subjected to motif searches in order to retrieve potential E2F elements. Both position and frequency of occurrence was determined using the publicly available execuTable of MatInspector (version 2.2) using matrices extracted from PlantCARE and matrices made especially for this particular analysis (Lescot et al., 2002). The relevance of each motif was evaluated against a background consisting of all the sequences from the dataset.

Results

To distinguish in the present data set the putative direct target genes of E2Fa-DPa from the secondary induced genes, the first 500 bp upstream of the ATG start codon of the genes with 2-fold or more change in expression was scanned for the presence of a E2F-like binding site matching the sequence (A/T)TT(G/C)(G/C)C(G/C)(G/C) (SEQ ID NO 2775). Of all the different permutations possible, only the TTTCCCGC (SEQ ID NO 2776) element was statistically enriched in the set of E2Fa-DPa upregulated genes, suggesting it is the preferred binding site of the E2Fa-DPa complex (Table 3). Moreover, target genes containing this element belong mainly to the group of genes involved in DNA replication and modification, being the main group of target genes in mammalian systems. These data illustrate that the TTTCCCGC sequence is the most likely cis element recognized by E2Fa-DPa. The observation that not all genes having this DNA sequence in their promoter suggests that the presence of the

TTTCCCCGC motif is not sufficient to make a gene responsive towards E2Fa-DPa, and that E2Fa-DPa co-operates with other factors to activate transcription.

It is not excluded that genes without an E2F-like-binding site are not directly activated by E2Fa-DPa. Chromatin immunoprecipitation experiments have shown that mammalian E2F factors can bind to promoters without a clear E2F recognition motif (Kiyosue *et al.*, 1994), suggesting that E2FDP might recognize non-canonical binding sites, or might be recruited by promoters through the association of other factors. In this respect, the *Chlorella vulgaris* nitrate reductase gene, of which the *Arabidopsis* homologue was shown herein to be induced by E2F-DPa, binds an E2F-DP complex, although a clear consensus binding site is lacking (Cannons and Shiflett, 2001).

E2F can activate as well as repress promoter activity.

In the Nicotiana benthamiana PCNA promoter a E2F sequence was identified acting as a negative regulatory element during development (Egelkrout et al., 2001). Also the tobacco ribonucleotide reductase small subunit gene contains a E2F element working as a repressor outside the S-phase (Chaboute et al., 2000). In the set of downregulated genes no particular enrichment of a specific E2F sequence could be seen (Table 3). Therefore the inventors believe that the E2Fa-DPa complex mainly works as a transcriptional activator, and that other E2F-DP complexes are involved in E2F-mediated transcriptional repression.

<u>Example 8: individual characterization of some genes identified by the method of the present invention</u>

The genes characterized hereunder, are particularly useful for making plants with improved growth characteristics. These preferred genes are introduced into a plant and upregulated or downregulated in order to simulate E2Fa/DPa effects and/or to alter one or more characteristics of a plant. The particular growth characteristic that may be influenced by these genes, is described in the following paragraphs by reference to the biological function of that particular gene.

At1g07000 showing homology to leucine zipper

At1g07000 is a potential leucine zipper that is not preceded by a basic domain. The leucine zipper consists of repeated leucine residues at every seventh position and mediates protein dimerization as a prerequisite for DNA-binding. The leucines are directed towards one side of an alpha-helix. The leucine side chains of two polypeptides are thought to interdigitate upon dimerization (knobs-into-holes model). The leucine zipper may dictate dimerization specificity.

Leucine zippers are DNA binding protein with dimerization properties, having important functions in development and stress tolerance in plants.

At1g09070 showing homology to Soybean Cold Regulate gene SRC2

This genes and its expressed protein is predicted in *Arabidopsis*, rice, corn, soybean, however, based on a homology search using the BLAST program, no functional homologue was known, not even a clear animal homologue, so no clear function can be predicted for this gene or protein (Takahashi,R. and Shimosaka,E. (1997)).

At1g21690 showing homology to Replication factor

Replication factor C (RFC) is a pentameric complex of five distinct subunits that functions as a clamp loader, facilitating the loading of proliferating cell nuclear antigen (PCNA) onto DNA during replication and repair. More recently the large subunit of RFC, RFC (p140), has been found to interact with the retinoblastoma (Rb) tumor suppressor and the CCAAT/enhancer-binding protein alpha (C/EBPalpha) transcription factor. It is reported that RFC (p140) associates with histone deacetylase activity and interacts with histone deacetylase 1 (HDAC1) (Anderson, L. A. and Perkins, N. D. (2002); Furukawa,T. et al. (2001)) RFC is poorly known in plants. It can be important for development for modulating gene expression during cell cycle at S phase, or through chromatin regulation.

At1g23030 showing homology to Armadillo protein

Members of the armadillo (arm) repeat family of proteins are implicated in tumorigenesis, embryonic development, and maintenance of tissue integrity. ARM proteins participate in linking cytoskeleton to membrane proteins and structures. These proteins share a central domain that is composed of a series of imperfect 45 amino acid repeats. Armadillo family members reveal diverse cellular locations reflecting their diverse functions. A single protein exerts several functions through interactions of its armadillo repeat domain with diverse binding partners. The proteins combine structural roles as cell-contact and cytoskeleton-associated proteins and signaling functions by generating and transducing signals affecting gene expression. The study of armadillo family members has made it increasingly clear that a distinction between structural proteins on the one hand and signaling molecules on the other is rather artificial. Instead armadillo family members exert both functions by interacting with a number of distinct cellular-binding partners. Proteins of the armadillo family are involved in diverse cellular processes in higher eukaryotes. Some of them, like armadillo, beta-catenin and plakoglobins have dual functions in intercellular junctions and signalling cascades. Others belonging to the importin-alpha-subfamily are involved in NLS (Nuclear localization signal) recognition and nuclear transport, while some members of the armadillo family have as yet

unknown functions. (Wang, Y. X. et al. (2001); Hatzfeld, M. (1999). ARM proteins are key protein binding units that are involved at several steps during development. Some are specific to the cell cycle APC degradation complex. These type of genes have been poorly studied in plants, some have been involved in light and gibberellin signaling in potato.

At1g27500 showing homology to Kinesin light chain.

The motor protein kinesin is a heterotetramer composed of two heavy chains of approximately 120 kDa and two light chains of approximately 65 kDa protein. Kinesin motor activity is dependent on the presence of ATP and microtubules. Conventional kinesin is prevented from binding to microtubules (MTs) when not transporting cargo. The function of LC kinesin is to keep kinesin in an inactive ground state by inducing an interaction between the tail and motor domains of HC; activation for cargo transport may be triggered by a small conformational change that releases the inhibition of the motor domain for MT binding. This protein is important to regulate movement controlled by microtubules within the cytoplasm, for example the flux of vesicles between the different cell membrane compartments.

At1g72180 showing homology to Putative receptor protein kinase

Plant receptor-like kinases (RLKs) are transmembrane proteins with putative amino-terminal extracellular domains and carboxyl-terminal intracellular kinase domains, with striking resemblance in domain organization to the animal receptor tyrosine kinases such as epidermal growth factor receptor. The recently sequenced *Arabidopsis* genome contains more than 600 RLK homologs. Although only a handful of these genes have known functions and fewer still have identified ligands or downstream targets, the studies of several RLKs such as CLAVATA1, Brassinosteroid Insensitive 1, Flagellin Insensitive 2, and S-locus receptor kinase provide much-needed information on the functions mediated by members of this large gene family. RLKs control a wide range of processes, including development, disease resistance, hormone perception, and self-incompatibility. Combined with the expression studies and biochemical analysis of other RLKs, more details of RLK function and signaling are emerging.

At1g72900 showing homology to Disease resistance protein (TIR virus resistance protein)

The TIR gene has been described by Kroczynska, B. et al. (1999).

At2g30590 showing homology to WRKY transcription factor (Toll/interleukin-1 receptor-like protein)

The sequence shows homology to tomato Cf-9 resistance gene Avr9/Cf-9 rapidly elicited protein 4 (NL27) (Hehl, R. et al. (1998)). WRKY proteins are a large group of transcription factors restricted to the plant kingdom. WRKY proteins are a recently identified class of DNA-binding proteins that recognize the TTGAC(C/T) W-box elements found in the promoters of a large number of plant defense-related genes (Dong and Chen, 2003). It has been found that the majority are responsive both to pathogen infection and to salicylic acid. The functions of all other WRKY genes revealed to date involve responses to pathogen attack, mechanical stress, and senescence (Dong and Chen, 2003).

At1g80530 showing homology to Nodulin

Infection of soybean roots by nitrogen-fixing Bradyrhizobium japonicum leads to expression of plant nodule-specific genes known as nodulins. Nodulin 26, a member of the major intrinsic protein/aquaporin (AQP) channel family, is a major component of the soybean symbiosome membrane (SM) that encloses the rhizobium bacteroid. These results indicate that nodulin 26 is a multifunctional AQP that confers water and glycerol transport to the SM, and likely plays a role in osmoregulation during legume/rhizobia symbioses (Dean et al. (1999). Rice (*Oryza sativa var. Nipponbare*) possesses two different homologues of the soybean early nodulin gene GmENOD93 (GmN93), OsENOD93a (homology of 58.2% to GmENOD93), OsENOD93b (homology of 42.3%). In intact rice tissues, OsENOD93b was most abundantly expressed in roots and at much lower levels in etiolated and green leaves, whereas the expression of OsENOD93a was very low in roots and etiolated leaves, and was not detected in green leaves. The level of OsENOD93a expression was enhanced markedly in suspension-cultured cells, whereas that of OsENOD93b did not increase (Reddy et al. (1998)). Homologues of genes that are produced in response to infection of soybean roots by bacteria are also present in other plants such rice. Their function is largely unknown, some functional homologues are identified such as a water channel involved in osmoregulation.

At2g34770 showing homology to Fatty acid hydroxylase

This gene has been described in Matsuda et al. (2001). A common feature of the membrane lipids of higher plants is a large content of polyunsaturated fatty acids, which typically consist of dienoic and trienoic fatty acids. Two types of omega-3 fatty acid desaturase, which are present in the plastids and in the endoplasmic reticulum (ER), respectively, are responsible for the conversion of dienoic to trienoic fatty acids. To establish a system for investigating the tissue-specific, and hormone-regulated expression of the ER-type desaturase gene (FAD3), transgenic plants of *Arabidopsis thaliana* (L.) Heynh. containing the firefly luciferase gene

(LUC) fused to the FAD3 promoter (FAD3::LUC) were constructed. The results as discussed in this report suggest that the expression of ER-type desaturase is regulated through synergistic and antagonistic hormonal interactions, and that such hormonal regulation and the tissue specificity of the expression of this gene are further modified in accordance with the growth phase in plant development (Wellesen K, et al. (2001); Kachroo P, et al. (2001); Kahn, R. A. et al. (2001); Smith, M. et al. (2000)).

At2g43402 showing homology to Cinnamoyl CoA reductase

CCR enzyme is involved in lignification. The CCR transcript is expressed in lignified organs, i.e. root and stem tissues, and is localized mainly in young differentiating xylem. Also, monolignols may be precursors of end products other than lignins. CCR catalyses the conversion of cinnamoyl-CoAs into their corresponding cinnamaldehydes, i.e. the first step of the phenylpropanoid pathway specifically dedicated to the monolignol biosynthetic branch. The two genes are differentially expressed during development and in response to infection. AtCCR1 is preferentially expressed in tissues undergoing lignification. In contrast, AtCCR2, which is poorly expressed during development, is strongly and transiently induced during the incompatible interaction with Xanthomonas campestris pv. Campestris leading to a hypersensitive response. Altogether, these data suggest that AtCCR1 is involved in constitutive lignification whereas AtCCR2 is involved in the biosynthesis of phenolics whose accumulation may lead to resistance (Lauvergeat et al. (2001)). This protein is involved during development, increase in growth diameter, lignification of vascular strands and interfascicular fibers.

At2g47440 showing homology to Tetratricopeptide repeat protein

The tetratricopeptide repeat (TPR) is found in many proteins performing a wide variety of functions, the TPR domain itself is believed to be a general protein recognition module. Different proteins may contain from 3 to 16 tandem TPR motifs (34 amino acid sequence). It has been shown that some proteins contain a TPR repeat are cell cycle regulated.

At3g23750 showing homology to Receptor like kinase TMK

The kinase domain of NtTMK1 contained all of 12 subdomains and invariant amino acid residues found in eukaryotic protein kinases. The extracellular domain contained 11 leucine-rich repeats, which have been implicated in protein-protein interactions. The amino acid sequence of NtTMK1 exhibited high homology with those of TMK1 of *Arabidopsis* and TMK of rice in both kinase and extracellular domains, suggesting that NtTMK1 is a TMK homologue of tobacco. The NtTMK1 transcripts were present in all major plant organs, but its level varied in different developmental stages in anthers and floral organs. NtTMK1 mRNA accumulation in

leaves was stimulated by CaCl2, methyl jasmonate, wounding, fungal elicitors, chitins, and chitosan. The NtTMK1 mRNA level also increased upon infection with tobacco mosaic virus (Cho and Pai (2000)). This protein is involved in different aspects of development and disease resistance.

At3g61460 showing homology to RING H2

RING-finger proteins contain cysteine-rich, zinc-binding domains and are involved in the formation of macromolecular scaffolds important for transcriptional repression and ubiquitination. RING H2 act as E3 ubiquitin-protein ligases and play critical roles in targeting the destruction of proteins of diverse functions in all eukaryotes, ranging from yeast to mammals. The *Arabidopsis* genome contains a large number of genes encoding RING finger proteins. A small group is constituted by more than 40 RING-H2 finger proteins that are of small size, not more than 200 amino acids, and contain no other recognizable protein-protein interaction domain(s). This type of genes is very important for several aspect of development, regulation of developmental proteins, cell cycle proteins.

At4g00730 showing homology to Homeodomain AHDP (antocyaninless 2)

This is a homeodomain transcription factor; similar to ATML1 and is very conserved and has epidermis specific expression. This sequence shows also homology to *Zea mays* mRNA for OCL3 protein (Ingram, G. C. et al. (2000)).

At4g13940 showing homology to adenosylhomocysteinase (Glutathione dependent formaldehyde dehydrogenase)

Glutathione-dependent formaldehyde dehydrogenase was described in Sakamoto, A. et al. (2002), *Arabidopsis* glutathione-dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase. S-Nitrosoglutathione (GSNO), an adduct of nitric oxide (NO) with glutathione, is known as a biological NO reservoir. Heterologous expression in *Escherichia coli* of a cDNA encoding a glutathione-dependent formaldehyde dehydrogenase of *Arabidopsis* thaliana showed that the recombinant protein reduces GSNO. The identity of the cDNA was further confirmed by functional complementation of the hypersensitivity to GSNO of a yeast mutant with impaired GSNO metabolism. This is the first demonstration of a plant GSNO reductase, suggesting that plants possess the enzymatic pathway that modulates the bioactivity and toxicity of NO.

At4g35050 showing homology to WD40 MSI3

Members of the MSI/RbAp sub-family of WD-repeat proteins are widespread in eukaryotic organisms and form part of multiprotein complexes that are involved in various biological

pathways, including chromatin assembly, regulation of gene transcription, and cell division. The *Zea mays* RbAp-like protein (ZmRbAp1) binds acetylated histones H3 and H4 and suppresses mutations that have a negative effect on the Ras/cAMP pathway in yeast. The ZmRbAp genes form a gene family and are expressed in different tissues of *Z. mays L.* plants. Determination of its expression pattern during maize seed development revealed that ZmRbAp transcripts are abundant during the initial stages of endosperm formation. In addition, the transcripts are specifically localized in shoot apical meristem and leaf primordia of the embryo. ZmRbAp genes play a role in early endosperm differentiation and plant development (Rossi et al. (2001)). Also Rb proteins are known to be involved in multi-protein complexes; there are Rb binding protein characterized; and Rb plays a role in chromatin remodeling and cell cycle control and is important in development and growth of organs. The retinoblastoma (RB) protein regulates G1 progression and functions through its association with various cellular proteins. Two closely related mammalian RB binding proteins, RbAp48 and RbAp46, share sequence homology with the Msi1 protein of yeast. MSI1 is a multicopy suppressor of a mutation in the IRA1 gene involved in the Ras-cAMP pathway that regulates cellular growth. Human RbAp48 is present in protein complexes involved in histone acetylation and chromatin assembly. Four plant RbAp48- and Msi1-like proteins have been identified: one from tomato, LeMSI1, and three from *Arabidopsis*. LeMSI1 can function as a multicopy suppressor of the yeast ira1 mutant phenotype. The LeMSI1 protein localizes to the nucleus and binds to a 65-kD protein in wild-type as well as ripening inhibitor (rin) and Neverripe (Nr) tomato fruit. LeMSI1 also binds to the human RB protein and the RB-like RRB1 protein from maize, indicating that this interaction is conserved between plants and animals (Ach et al. (1997)).

At4g36670 showing homology to Sugar transporter

The ERD6 clone is expressed after exposure to dehydration stress for 1 h. The ERD6 is related to sugar transporters of bacteria, yeasts, plants and mammals. Hydropathy analysis revealed that ERD6 protein has 12 putative transmembrane domains and a central hydrophilic region. Sequences that are conserved at the ends of the 6th and 12th membrane-spanning domains of sugar transporters are also present in ERD6. ERD6 gene is a member of a multigene family in the *Arabidopsis* genome. The expression of the ERD6 gene was induced not only by dehydration but also by cold treatment (Kiyosue et al. (1998)) .

At5g01870 showing homology to Lipid transfer protein

Nonspecific lipid transfer proteins (LTPs) from plants are characterized by their ability to stimulate phospholipid transfer between membranes *in vitro*. However, because these proteins are generally located outside of the plasma membrane, it is unlikely that they have a similar role *in vivo*. The LTP1 promoter was active early in development in protoderm cells of

embryos, vascular tissues, lignified tips of cotyledons, shoot meristem, and stipules. In adult plants, the gene was expressed in epidermal cells of young leaves and the stem. In flowers, expression was observed in the epidermis of all developing influorescence and flower organ primordia, the epidermis of the siliques and the outer ovule wall, the stigma, petal tips, and floral nectaries of mature flowers, and the petal/sepal abscission zone of mature siliques. Consistent with a role for the LTP1 gene product in some aspect of secretion or deposition of lipophilic substances in the cell walls of expanding epidermal cells and certain secretory tissues. The LTP1 promoter region contained sequences homologous to putative regulatory elements of genes in the phenylpropanoid biosynthetic pathway, suggesting that the expression of the LTP1 gene may be regulated by the same or similar mechanisms as genes in the phenylpropanoid pathway (Thoma, S. et al. (1994)). More background knowledge to this type of genes can be found in the following references: Clark, A. M. et al., (1999); Toonen, M. A. et al. (1997); Molina, A. (1997); Thoma, S. et al. (1994).

At5g02820 showing homology to SPO like

Plant steroid hormones, such as brassinosteroids (BRs), play important roles throughout plant growth and development. Plants defective in BR biosynthesis or perception display cell elongation defects and severe dwarfism. Two dwarf mutants named bin3 and bin5 with identical phenotypes to each other display some characteristics of BR mutants and are partially insensitive to exogenously applied BRs. In the dark, bin3 or bin5 seedlings are de-etiolated with short hypocotyls and open cotyledons. Light-grown mutant plants are dwarfs with short petioles, epinastic leaves, short inflorescence stems, and reduced apical dominance. BIN3 and BIN5 were cloned and show that BIN5 is one of three putative *Arabidopsis* SPO11 homologs (AtSPO11-3) that also shares significant homology to archaebacterial topoisomerase VI (TOP6) subunit A, whereas BIN3 represents a putative eukaryotic homolog of TOP6B. The pleiotropic dwarf phenotypes of bin5 establish that, unlike all of the other SPO11 homologs that are involved in meiosis, BIN5/AtSPO11-3 plays a major role during somatic development. Furthermore, microarray analysis of the expression of about 5500 genes in bin3 or bin5 mutants indicates that about 321 genes are down-regulated in both of the mutants, including 18 of 30 BR-induced genes. These results suggest that BIN3 and BIN5 may constitute an *Arabidopsis* topoisomerase VI that modulates expression of many genes, including those regulated by BRs (Yin Y et al. (2002)). More background information on this type of gene can be found in the following references: Soustelle, C. et al. (2002); Kee, K. and Keeney, S. (2002); Hartung, F. and Puchta, H. (2001); Grelon, M. et al. (2001).

At5g14420 showing homology to copine I (phospholipid binding protein)

The copines are a newly identified class of calcium-dependent, phospholipid binding proteins that are present in a wide range of organisms, including *Paramecium*, plants, *Caenorhabditis elegans*, mouse, and human. However, the biological functions of the copines are unknown. A humidity-sensitive copine mutant was made in *Arabidopsis* and under non-permissive, low-humidity conditions, the cpn1-1 mutant displayed aberrant regulation of cell death that included a lesion mimic phenotype and an accelerated hypersensitive response (HR). However, the HR in cpn1-1 showed no increase in sensitivity to low pathogen titers. Low-humidity-grown cpn1-1 mutants also exhibited morphological abnormalities, increased resistance to virulent strains of *Pseudomonas* syringae and *Peronospora parasitica*, and constitutive expression of pathogenesis-related (PR) genes. Growth of cpn1-1 under permissive, high-humidity conditions abolished the increased disease resistance, lesion mimic, and morphological mutant phenotypes but only partially alleviated the accelerated HR and constitutive PR gene expression phenotypes. The disease resistance phenotype of cpn1-1 suggests that the CPN1 gene regulates defense responses. Alternatively, the primary function of CPN1 may be the regulation of plant responses to low humidity, and the effect of the cpn1-1 mutation on disease resistance may be indirect (Jambunathan et al. (2001)). *Arabidopsis* growth over a wide range of temperatures requires the BONZAI1 (BON1) gene because bon1 null mutants make miniature fertile plants at 22°C but have wild-type appearance at 28°C. The expression of BON1 and a BON1-associated protein (BAP1) is modulated by temperature. Thus BON1 and BAP1 may have a direct role in regulating cell expansion and cell division at lower temperatures. BON1 contains a Ca(2+)-dependent phospholipid-binding domain and is associated with the plasma membrane. It belongs to the copine gene family, which is conserved from protozoa to humans. The data here obtained suggest that this gene family may function in the pathway of membrane trafficking in response to external conditions (Hua et al. (2001)). The major calcium-dependent, phospholipid-binding protein obtained from extracts of *Paramecium tetraurelia*, named copine, had a mass of 55 kDa, bound phosphatidylserine but not phosphatidylcholine at micromolar levels of calcium but not magnesium, and promoted lipid vesicle aggregation. Current sequence databases indicate the presence of multiple copine homologs in green plants, nematodes, and humans. The full-length sequences reveal that copines consist of two C2 domains at the N terminus followed by a domain similar to the A domain that mediates interactions between integrins and extracellular ligands. The association with secretory vesicles, as well the general ability of copines to bind phospholipid bilayers in a calcium-dependent manner, suggests that these proteins may function in membrane trafficking (Creutz et al. (1998)).

At5g49160 showing homology to cytosine methyltransferase

DNMT3L is a regulator of imprint establishment of normally methylated maternal genomic sequences. DNMT3L shows high similarity to the de novo DNA methyltransferases, DNMT3A and DNMT3B, however, the amino acid residues needed for DNA cytosine methyltransferase activity have been lost from the DNMT3L protein sequence. Apart from methyltransferase activity, Dnmt3a and Dnmt3b serve as transcriptional repressors associating with histone deacetylase (HDAC) activity. DNMT3L can also repress transcription by binding directly to HDAC1 protein. PHD-like zinc finger of the ATRX domain is the main repression motif of DNMT3L, through which DNMT3L recruits the HDAC activity needed for transcriptional silencing. DNMT3L as a co-repressor protein and suggest that a transcriptionally repressed chromatin organisation through HDAC activity is needed for establishment of genomic imprints (Aapola et al. (2002)). More background information on this type of gene can be found in Chen, T. et al. (2002); Bartee, L. and Bender, J. (2001); Freitag M. et al. (2002). In *Arabidopsis* a SWI2/SNF2 chromatin remodeling factor-related protein DDM1 and a cytosine methyltransferase MET1 is required for maintenance of genomic cytosine methylation. Mutations in either gene cause global demethylation. There are also effects of these mutations on the PAI tryptophan biosynthetic gene family, which consists of four densely methylated genes arranged as a tail-to-tail inverted repeat plus two unlinked singlet genes. The methylation mutations caused only partial demethylation of the PAI loci: ddm1 had a strong effect on the singlet genes but a weaker effect on the inverted repeat, whereas met1 had a stronger effect on the inverted repeat than on the singlet genes. The double ddm1 met1 mutant also displayed partial demethylation of the PAI genes, with a pattern similar to the ddm1 single mutant. To determine the relationship between partial methylation and expression for the singlet PAI2 gene a novel reporter strain of *Arabidopsis* was constructed, in which PAI2 silencing could be monitored by a blue fluorescent plant phenotype diagnostic of tryptophan pathway defects. This reporter strain revealed that intermediate levels of methylation correlate with intermediate suppression of the fluorescent phenotype. Other background information can be found in Finnegan, E. J. and Kovac K. A. (2000). Plant DNA methyltransferases. DNA methylation is an important modification of DNA that plays a role in genome management and in regulating gene expression during development. Methylation is carried out by DNA methyltransferases which catalyse the transfer of a methyl group to bases within the DNA helix. Plants have at least three classes of cytosine methyltransferase which differ in protein structure and function. The METI family, homologues of the mouse Dnmtl methyltransferase, most likely function as maintenance methyltransferases, but may also play a role in *de novo* methylation. The chromomethylases, which are unique to plants, may preferentially methylate DNA in heterochromatin; the remaining class, with similarity to Dnmt3 methyltransferases of mammals, are putative *de novo* methyltransferases. The various classes of methyltransferase

may show differential activity on cytosines in different sequence contexts. Chromomethylases may preferentially methylate cytosines in CpNpG sequences while the *Arabidopsis* METI methyltransferase shows a preference for cytosines in CpG sequences. Additional proteins, for example DDM1, a member of the SNF2/SWI2 family of chromatin remodeling proteins, are also required for methylation of plant DNA.

At5g54940 showing homology to Translation initiation factor (translational initiation factor eIF1),

Protein synthesis has not been considered to be fundamental in the control of cell proliferation. However, data are emerging on the involvement of this process in cell growth and tumorigenesis. Protein biosynthesis is a central process in all living cells. It is one of the last steps in the transmission of genetic information stored in DNA on the basis of which proteins are produced to maintain the specific biological function of a given cell. Protein synthesis takes place on ribosomal particles where the genetic information transcribed into mRNA is translated into protein. The process of protein synthesis on the ribosome consists of three phases: initiation, elongation and termination. Brassinosteroids (BRs) regulate the expression of numerous genes associated with plant development, and require the activity of a Ser/Thr receptor kinase to realize their effects. In animals, the transforming growth factor-beta (TGF-beta) family of peptides acts via Ser/Thr receptor kinases to have a major impact on several pathways involved in animal development and adult homeostasis. TGF-beta receptor-interacting protein (TRIP-1) was previously shown by others to be an intracellular substrate of the TGF-beta type II receptor kinase which plays an important role in TGF-beta signaling. TRIP-1 is a WD-repeat protein that also has a dual role as an essential subunit of the eukaryotic translation initiation factor eIF3 in animals, yeast and plants, thereby revealing a putative link between a developmental signaling pathway and the control of protein translation. In yeast, expression of a TRIP-1 homolog has also been closely associated with cell proliferation and progression through the cell cycle. Transcript levels of TRIP-1 homologs in plants are regulated by BR treatment under a variety of conditions, and transgenic plants expressing antisense TRIP-1 RNA exhibit a broad range of developmental defects, including some that resemble the phenotype of BR-deficient and -insensitive mutants. This correlative evidence suggests that a WD-domain protein with reported dual functions in vertebrates and fungi might mediate some of the molecular mechanisms underlying the regulation of plant growth and development by BRs (Jiang and Clouse (2001)). The *Arabidopsis* COP9 signalosome is a multisubunit repressor of photomorphogenesis that is conserved among eukaryotes. This complex may have a general role in development. association between components of the COP9 signalosome (CSN1, CSN7, and CSN8) and two subunits of eukaryotic translation initiation factor 3 (eIF3), eIF3e (p48, known also as INT-6) and eIF3c

(p105). AteIF3e coimmunoprecipitated with CSN7, and eIF3c coimmunoprecipitated with eIF3e, eIF3b, CSN8, and CSN1. eIF3e directly interacted with CSN7 and eIF3c. eIF3e and eIF3c are probably components of multiple complexes and that eIF3e and eIF3c associate with subunits of the COP9 signalosome, even though they are not components of the COP9 signalosome core complex. This interaction may allow for translational control by the COP9 signalosome (Yahalom et al. (2001)).

At5g56740 showing homology to Histone acetyl transferase HATB

Transforming viral proteins such as E1A which force quiescent cells into S phase have two essential cellular target proteins, Rb and CBP/p300. Rb regulates the G1/S transition by controlling the transcription factor E2F. CBP/p300 is a transcriptional co-activator with intrinsic histone acetyl-transferase activity. This activity is regulated in a cell cycle dependent manner and shows a peak at the G1/S transition. CBP/p300 is essential for the activity of E2F, a transcription factor that controls the G1/S transition. It was found that CBP HAT activity is required both for the G1/S transition and for E2F activity. Thus CBP/p300 seems to be a versatile protein involved in opposing cellular processes, which raises the question of how its multiple activities are regulated (Ait-Si-Ali, S. et al (2000)). The BRCA2 is a histone acetyltransferase. Two potential functions of BRCA2 were proposed which includes role in the regulation of transcription and also in DNA repair. Forty-five-amino acid region encoded by exon 3 of BRCA2 was shown to have transcriptional activation function. Recent studies of the several enzymes involved in acetylation and deacetylation of histone residues have revealed a possible relationship between gene transcriptional activation and histone acetylation. Since BRCA2 appear to function as a transcriptional factor, Histone acetyl transferase (HAT) activity of BRCA2 was tested. Also, evidence that BRCA2 has intrinsic HAT activity, which maps to the amino-terminal region of BRCA2, was presented. It was demonstrated that BRCA2 proteins acetylate primarily H3 and H4 of free histones. These observations suggest that HAT activity of BRCA2 may play an important role in the regulation of transcription and tumor suppressor function (Siddique et al. (1998)). These types of genes are very important for regulation of genes involved in development, cell cycle control, and chromatin structure.

At5g61520 showing homology to STP3 sucrose transporter

For developing seeds of grain legumes, photoassimilates released to the seed apoplasm from maternal seed coats are retrieved by abaxial epidermal and subepidermal cells (dermal cell complexes) of cotyledons followed by symplasmic passage to their underlying storage parenchyma cells. In some species, the cells of these complexes differentiate into transfer cells (e.g. broad bean and pea, Patrick and Offler,2001). Sucrose is a major component of the photoassimilates delivered to cotyledons (Patrick and Offler, 2001; Weber et al., 1997b).

Sucrose transporter (SUT) genes have been cloned, and functionally characterized as sucrose/H+ symporters, from developing cotyledons of broad bean (VfSUT1, Weber et al., 1997a) and pea (PsSUT1, Tegeder et al., 1999). SUTs and P-type H+-ATPases have been shown to co-localize to plasma membranes of dermal cell complexes in developing cotyledons of broad bean (Harrington et al., 1997; Weber et al., 1997a) and French bean (Tegeder et al., 2000). In contrast, for pea cotyledons, SUT is also present in storage parenchyma cells, but is 4-fold less active than SUT(s) localized to epidermal transfer cells (Tegeder et al., 1999). These type of genes are Important for seed filling.

At5g66210 showing homology to Calcium dependent protein kinase

In plants, numerous Ca(2+)-stimulated protein kinase activities occur through calcium-dependent protein kinases (CDPKs). These novel calcium sensors are likely to be crucial mediators of responses to diverse endogenous and environmental cues. However, the precise biological function(s) of most CDPKs remains elusive. The *Arabidopsis* genome is predicted to encode 34 different CDPKs. The *Arabidopsis* CDPK gene family was analyzed and the expression, regulation, and possible functions of plant CDPKs was reviewed. By combining emerging cellular and genomic technologies with genetic and biochemical approaches, the characterization of *Arabidopsis* CDPKs provides a valuable opportunity to understand the plant calcium-signaling network (Cheng et al., 2002). These type of genes are Important for stress signaling.

At2g25970 showing homology to KH RNA binding domain

Lorkovic and Barta (2002) described RNA recognition motif (RRM) and K homology (KH) domain RNA-binding proteins from the flowering plant *Arabidopsis* thaliana. The most widely spread motifs are the RNA recognition motif (RRM) and the K homology (KH) domain. The *Arabidopsis* genome encodes 196 RRM-containing proteins, a more complex set than found in *Caenorhabditis elegans* and *Drosophila melanogaster*. In addition, the *Arabidopsis* genome contains 26 KH domain proteins. Most of the *Arabidopsis* RRM-containing proteins can be classified into structural and/or functional groups, based on similarity with either known metazoan or *Arabidopsis* proteins. Approximately 50% of *Arabidopsis* RRM-containing proteins do not have obvious homologues in metazoa, and for most of those that are predicted to be orthologues of metazoan proteins, no experimental data exist to confirm this. Additionally, the function of most *Arabidopsis* RRM proteins and of all KH proteins is unknown. The higher complexity of RNA-binding proteins in *Arabidopsis*, as evident in groups of SR splicing factors and poly(A)-binding proteins, may account for the observed differences in mRNA maturation between plants and metazoa. The function of this type of genes is largely unknown, but could

be related to PUMILIO genes from *Drosophila*. Important for regulation of gene expression at the post-transcriptional level, role in development, stress tolerance.

At3g07800 showing homology to Thymidine kinase

This type of thymidine kinase genes is cell cycle regulated, E2F regulated, is responsible for production of thymidine triphosphate. This type of gene plays a role as a precursor for DNA synthesis and is therefore a marker of S phase.

At5g47370 showing homology to Homeobox leucine zipper protein .

This type of homeobox genes is important for development and growth and also for stress tolerance. At5g47370 is homeobox-leucine zipper protein HAT2 (HD-ZIP protein 2). Homeobox genes are known as transcriptional regulators that are involved in various aspects of developmental processes in many organisms. Homeodomain transcription factors regulate fundamental body plan of plants during embryogenesis, as well as morphogenetic events in the shoot apical meristem (SAM) after embryogenesis. HOX1 belongs to the subset of homeodomain leucine zipper (HD-zip) and is involved in the regulation of vascular development (Scarpella *et al.*, 2000; Meijer *et al.*, 2000). The sequences for the rice OsHOX1 orthologue are deposited in Genbank under the accession number X96681 (cDNA) and CAA65456.2 (protein), which sequences are both herein incorporated by reference.

BAA23337.1 OsMYB1

MYB-like DNA binding proteins are involved in the control of specific developmental steps in different organs. OSMYB1 binds to a seed specific element in the seed storage protein glutelin, is expressed in endosperm of rice seeds, and plays an important role during seed maturation (Suzuki *et al.*, 1997).

BAA89798 OsNAC4

NAC domain containing genes, such as NO APICAL MERISTEM in petunia and CUP-SHAPED COTYLEDON2 and NAP in *Arabidopsis*, have crucial functions in plant development (Kikuchi *et al.*, 2000). These genes are involved in the control of organ primordium delimitation and lateral organ development. It has also been recently shown that a member of the NAC family of transcription factor can induces formation of ectopic shoots on cotyledons (Daimon *et al.*, 2003).

AAD37699 rice homeodomain leucine zipper protein HOX6 (partial)

Homeobox genes are known as transcriptional regulators that are involved in various aspects of developmental processes in many organisms. Homeodomain transcription factors regulate

fundamental body plan of plants during embryogenesis, as well as morphogenetic events in the shoot apical meristem (SAM) after embryogenesis. HOX6 is a homologue of the *Arabidopsis* homeobox gene Athb-12 (Lee *et al.*, 2001). Athb-12 is a transcriptional activator important in regulating certain developmental processes as well as in the plant's response to water stress involving ABA-mediated gene expression. At3g61890 is the *Arabidopsis* sequence corresponding with the rice HOX6 sequence of AAD37699.

AK104073 OsMYB predicted

This gene is homologous to the *Arabidopsis* gene CIRCADIAN CLOCK ASSOCIATED (CCA1) gene that encodes a related MYB transcription factor, which regulates circadian rhythms (Carre et Kim, 2002). This gene as well as the MYB homologue, regulate the period of circadian rhythms in gene expression and leaf movements.

Example 9: NMR study of E2Fa/DPa overexpressing plants.

In support of the microarray studies identifying the increased or decreased expression level of E2F-target genes in E2Fa-DPa overexpressing plants, the effects of E2Fa/DPa overexpression on the protein level and ultimately on the level of metabolites were studied via the techniques of metabolomics. Metabolomics means qualitative and quantitative analysis of the metabolites present at a certain time in a cell culture or a whole biological tissue. Metabolites, as designated here, are small molecular weight molecules (typically under 1000 Daltons), of which many are already known (such as urea, lipids, glucose or certain small hormones) while others are still to be identified. Metabolites are the final product of the protein content of the cell. The main methods used to detect and quantify of those molecules are mass spectrometry or NMR spectroscopy (Nicholson *et al.*, 2002) after extraction and purification of the metabolites from the organism.

Now NMR spectroscopy on whole organisms has been performed. The recording of spectra of the metabolites was possible without any prior purification of the plant material. Hereto, the samples were spun at the magic angle. This technique, dubbed "High Resolution Magic Angle Spinning" (HRMAS) NMR, has now been used on intact plantlets. 1H-13C HSQC spectra were recorded on intact wild-type and E2Fa/DPa overexpressing plantlets of *Arabidopsis thaliana*, and monitored the changes in metabolite pattern. From the spectra, a shift in the metabolome of E2Fa/DPa overexpressing plants when compared to wild-type plants, was observed. These spectra are processed in order to map the observed metabolic differences.

Example 10: molecular and phenotypic analysis of Arabidopisis plants transformed with the genes according to the present invention

Arabidopsis thaliana plants are transformed with at least one of the genes of the present invention as presented in Table 4 or 5, operably linked to a plant promoter.

In one example, *Arabidopsis* plants were transformed with the genes as presented in Table 6. The vectors used ware derived from the expression vector pK7WGD2, carrying the CaMV35S promoter for expression of the gene. For transformation, the flower dip method described by Bechtold and Pelletier (1998) was used.

Table 6: Genes that were selected and transformed into *Arabidopsis*

CODE	AGI	GENE	PRIMERS	PCR	pDON R207	PK7WGD2	Flower dip
1	At1g33960	AIG1	282 + 283				
2	At1g21690	Putative replication factor	284 + 285	OK	OK		
3	At3g23250	Myb transcription factor	286 + 287				
4	At5g08450	Unknown	288 + 289	OK	OK	OK(clone1)	OK
5	At3g45730	Unknown	290 + 291	OK	OK	OK(clone4)	OK
6	At1g56150	Unknown	292 + 293				
7	At5g66580	Unknown	294 + 295	OK	OK	OK	OK
8	At4g33050	Unknown	296 + 297	OK	OK	OK	OK
9	At1g76970	Unknown	298 + 299	OK	partieel	OK(clone4)	
10	At2g41780	Unknown	300 + 301	OK	OK	OK(clone1)	OK
11	At5g14530	WD40 repeat protein	302 + 303	OK	OK	OK	OK
A	At3g02550	Unknown	310 + 311	OK	OK	OK(A10.7)	OK
B	At5g47370	homeobox-leucine zipper protein-like	312 + 313	OK	OK	OK(clone4)	OK
C	At1g57680	Unknown	314 + 315				OK
D	At1g07000	leucine zipper-containing protein	316 + 317	OK	OK		OK
E	At2g22430	homeodomain TF Athb-6	318 + 319	OK	OK	OK(clone1)	OK
F	At4g28330	Unknown	320 + 321	OK	OK	OK(clone4)	OK
G	At3g23750	receptor kinase	322 + 323	OK	OK		
H	At5g66210	Ca-dep kinase	324 + 325	OK	OK	OK(clone2)	OK
I	At4g02680	Unknown	326 + 327			OK(clone4)	OK
J	At2g30590	worky74	328 + 329	OK	OK	OK(clone2)	OK
K	At2g46650	Unknown	330 + 331				
L	At2g47440	Unknown	332 + 333	OK	OK	OK(clone5)	OK
M	At2g15510	Unknown	334 + 335				

12	At5g56740	Histone acetylase HAT B	348 + 349	OK	OK	OK	OK
13	At3g24320	Putative mismatch binding protein	350 +351	OK	OK	OK (13,4)	OK
14	At4g00730	Anthocyaninless2	352 + 353				
15	At1g23030	arm-repeat containing protein	354 + 355	OK	OK	OK(clone11)	OK
16	At5g54380	receptor-protein kinase-like protein	356 + 357				
17	At1g72180	putative leucine-rich receptor-like protein kinase	358 + 359	OK	OK	OK	OK
18	At1g61100	Unknown	360 + 361	OK	OK	OK (18,1)	OK
19	At2g25970	Unknown	362 + 363	OK	OK	OK	OK
20	At2g38310	Unknown	364 + 365	OK	OK	OK	OK
21	At3g45970	Unknown	366 + 367	OK	OK	OK (21,3)	OK

Code: internal reference code of the gene; AGI: accession number of the protein in the internal dataset, here with reference to the MIPS database accession number; Gene: name of the protein; primers: PCR primers used to isolate the ORF of the gene by RT-PCR using cDNA; prepared form E2Fa-DPa overexpressing plants; PCR: PCR completed successfully; pDONR207: cloning in this vector completed (www.invitrogen.com); pK7WGD2: cloning of the genes in the vector under control of the CaMV 35S promoter (Karimi et al., Trends Plant Sci. 2002 May;7(5):193-5); Flower dip: transformation of Arabidopsis plants with the pK7WGD2 vector.

The transformed *Arabidopsis* plants are evaluated as described below.
After molecular analysis (PCR, RT-PCR, Western-blot, southern-blot, Northern blot, NMR), the plants with modified E2F target gene expression levels, are submitted to phenotypic analysis. Special attention is given to root growth and leaf development.

The root of *A. thaliana*, which has a rather constant diameter and rather uncomplicated radial symmetry, is a perfect model system for studying and determining the effects of modulation of expression levels of an E2F-target on an intact, growing tissue.

The root of *A. thaliana* comprises a thick unicellular layer of the epidermis cells, one of cortex cells, one of endodermis cells and one of pericyclus cells that circumvent the vascular tube. Because of its transparency, the root of *A. thaliana*, these cellular layers can be visualized by interference contrast microscopy. By this means the origin of the cells in a specific cell layer can be traced back to a set of dividing mother cells in the meristem (Dolan *et al.*, 1993). By measurement of the cell length of a specific cell layer in function of the distance to the root tip,

and the rate of movement of the cells away from the root tip (measured via time-laps photography), it is possible to determine the contribution of both the cell elongation as well as of cell division to the total root growth (Beemster and Baskin, 1998).

The effects of the E2F-target overexpression in the leaves is determined via microscopic techniques after clearance of the leaves of lactic acid. This analysis is performed on the first developed leaf pear, since this leaf pear is most comparable between different plants. By measurement of the cell number and the number of epidermal cells at different time points during leaf development, it is deduced when the leaf cells stop to divide, when they start to differentiate, the duration of their cell cycle is, and their final cell size (De Veylder *et al.*, 2001 a and b). Moreover, this method allows the analysis of the effect of E2F target overexpression on the formation of stomata.

The effect of the E2F-target overexpression is also studied via biochemical means. Functional assays are developed for the specific enzymatic activity of the studied E2F-target gene. These functional methods are based on expression of a reported gene in case the E2F-target is in itself a transcription activator or repressor. Functional assays are based on the incorporation of radioactive nitrogen or radioactive carbon or other radiolabelled metabolites when the enzyme is involved in the nitrogen or carbon metabolisms or other processes involving metabolites. By the comparison of the incorporated radioactivity between the control line and the transgenic line, the enzymatic activity of the E2F-target can be measured.

Functional assays are based on the incorporation of radioactive ATP, radioactive purines or pyrimidines when the enzyme is involved in DNA replication and/or modification. Functional assays are based on labeled carbohydrates when the enzyme is involved in cell wall biogenesis, or ATP when the enzyme is involved in processes of the chloroplast, or calcium when the enzyme is involved in signal transduction.

In *A. thaliana,* besides the mitotic cell cycle also an alternative cell cycle is observed, in which DNA is replicated in the absence of mitosis or cytokinesis. This so-called endoreduplication process occurs often in plants. Until today, the physiological significance of endoreduplication is unknown. Possibly, it is a mechanism to increase the number of DNA copies per cell, which allows more transcription. In support of this hypothesis, endoreduplication often occurs in cells with high metabolic activity (Nagl, 1976). However, as a consequence of endoreduplication the cells are bigger, which is especially useful for increasing yield of cytoplasmatic component, for example storage proteins of the seed cells.

To study the effects of E2F-target overexpression on the process of endoreduplication, the DNA content of the control plants and the transgenic plant is measured via flow-cytometry. A more detailed analysis is obtained by measuring the DNA content of individual cells colored with DNA-binding fluorochrome (e.g. DAPI). The intensity of the color of the nucleus is in proportion with its DNA content. Relative DNA-measurements can be obtained via a microdensitometer. This technique allows determining a specific tissue the endoreduplication pattern of the transgenic plants.

Example 11: Use of the invention in corn

The invention described herein can also be used in maize. To this aim, a gene according to the present invention as presented in Table 4 or 5, for example a gene selected from Tables 1 or 2, or a gene selected from the group described in Example 8, or a gene selected from the group presented in Table 6 or 7, or a homologue thereof such as for example a maize ortholog or a rice ortholog, is cloned under control of a promoter operable in maize, in a plant transformation vector suited for *Agrobacterium*-mediated transformation of corn. These constructs are designed for overexpression or for downregulation. In a series of experiments, genes selected from Table 5 (downregulated in E2Fa/DP transgenics) are overexpressed in transgenic corn and genes selected from Table 4 (upregulated in E2Fa-DPa overexpressing plants) are downregulated in transgenic corn. Suitable promoter for driving expression of the genes of the present invention are as presented in Tables I, II, III and IV or in Table V.

Suitable promoter for driving expression of the genes of the present invention in corn are the rice GOS2 promoter or any other promoter as mentioned herein above. Vectors useful for expression of one or more E2F targets according to the present invention are standard binary vectors, such as the pPZP vector described in Hajdukiewicz *et al.* ((1994) Plant Mol Biol 25: 989-994) or a superbinary vector. Vectors and methods to use *Agrobactorium*-mediated transformation of maize have been described in literature (Ishida *et al.*, Nat Biotechnol. 1996 Jun;14(6):745-50; Frame *et al.*, Plant Physiol. 2002 May;129(1):13-22) and are herein incorporated by reference. Transgenic plants made by these methods are grown in the greenhouse for T1 seed production. Inheritability and copy number of the transgene are checked by quantitative real-time PCR and Southern blot analysis and expression levels of the transgene are determined by reverse PCR and Northern analysis. Transgenic lines with single copy insertions of the transgene and with varying levels of transgene expression are selected for T2 seed production. Progeny seeds are germinated and grown in the greenhouse in conditions well adapted for maize (16:8 photoperiod, 26-28°C daytime temperature and 22-24°C nighttime temperature) as well under water-deficient, nitrogen-deficient, and excess NaCl conditions. Null segregants from the same parental line, as well as wild type plants of the same

cultivar are used as controls. The progeny plants resulting from the selfing or the crosses are evaluated on different growth parameters, such as biomass and developmental parameters. These parameters include stem size, number of leaves, total above ground area, leaf greenness, time to maturity, flowering time, time to flower, ear number, harvesting time. The seeds of these lines are also checked on various parameters, such as grain size, total grain yield (number and/or weight) per plant, and grain quality (starch content, protein content and oil content). Lines that are most significantly improved versus the controls for any of the above-mentioned parameters are selected for further field-testing and marker-assisted breeding, with the objective of transferring the field-validated transgenic traits into commercial germplasm. Methods for testing maize for growth and yield-related parameters in the field are well established in the art, as are techniques for introgressing specific loci (such as transgene containing loci) from one germplasm into another. Corn plants according to the present invention have changed growth characteristics compared to the wild-type plants, such as for example any one or more of increased biomass, increased yield, increased number and/or size of organs (including seeds), increased harvest index, increased rate of growth and/or development (e.g. decreased cycling time, decreased time to harvest, early flowering), increased tolerance to environmental stress conditions (e.g. tolerance to salt, drought and/or cold).

Example 12: Rice transformation with the genes according to the present invention

In a particular example of the present invention, the genes as identified above in Tables 4 and 5, or an orthologue from another plant, for example the rice orthologue, is transformed into rice. In particular, the genes as presented in Tables 6 and 7, or the rice orthologues are cloned into a plant expression vector operably linked to a promoter for overexpression or downregulation of these genes.

The genes as represented in Table 7 are cloned into a plant expression vector operably linked to a GOS2 promoter for overexpression or downregulation. For overexpression these genes are cloned in the sense orientation and for downregulation a hairpin construct as described in Wesley *et al.* (2001) is made. Other promoters that are used to drive expression of these genes are other constitutive promoters, such as for example the ubiquitin promoter or PRO170 (high mobility group protein), or PRO61 (beta expansin promoter). Also tissue specific promoters are used to drive expression of the genes of the present invention in rice, such as for example promoters specific for meristem (PRO120: metallothionein), or vegetative tissue (PRO123: protochlorophyllid reductase), PRO173: cytoplasmic malate deshydrogenase); or endosperm (PRO90: prolamin, PRO135: alpha globulin), or embryo PRO218: oleosin, PRO151: WSI18, PRO200: OSH1, PRO175: RAB21; or the whole seed (PRO58: proteinase

inhibitor), or any other promoter described herein above. The vectors used are plant transformation vector suited for *Agrobacterium*-mediated transformation of rice, such as for example binary vectors of the pCAMBIA type or super binary vectors. Such vectors and methods for rice transformation have been described in literature by Aldemita and Hodges (1996) Chan *et al.* (1993), Hiei *et al.* (1994) or in EP1198985 and which teachings herein incorporated by reference.

Table 7: genes (presented by their encoded proteins) selected for rice transformation

>CDS3435 NP_176081.1 At1g57680 (*Arabidopsis*) MPLTKLVPDAFGVVTICLVALLVLLGLLCIAYSFYFQSHVRKQGYIQLGY FSGPWIIRITFILFAIWWAVGEIFRLSLLRRHRRLLSGLDLRWQENVCKW YIVSNLGFAEPCLFLTLMFLLRAPLKMESGALSGKWNRDTAGYIILYCLP MLALQLAVVLSESRLNGGSGSYVKLPHDFTRTYSRVIIDHDEVALCTYP LLSTILLGVFAAVLTAYLFWLGRQILKLVINKRLQKRVYTLIFSVSSFLPLR IVMLCLSVLTAADKIIFEALSFLAFLSLFCFCVVSICLLVYFPVSDSMALRG LRDTDDEDTAVTEERSGALLLAPNSSQTDEGLSLRGRRDSGSSTQERY VELSLFLEAEN	SEQ ID NO 1 and 2
>CDS3436 BAC42858.1 At3g45730 (*Arabidopsis*) MELPSPYSSRKEESTVPPKRGRVKIMIFRDLVRSETSMAPTPRRGRIKK MIAGDLVGSGKQNNYDGDGKRGG	SEQ ID NO 3 and 4
>CDS3449 BAA23337.1 OS MYB1 (Rice) MGRSPCCEKAHTNKGAWTKEEDQRLIAYIRAHGEGCWRSLPKAAGLL RCGKSCRLRWMNYLRPDLKRGNFTDDEDELIIRLHSLLGNKWSLIAGQL PGRTDNEIKNYWNTHIKRKLLARGIDPQTHRPLLSGGDGIAASNKRHHR RRIPYPSRRRRRPRRSSPCEAAAAAAPGRLLGRRLPQQQRHNEHGGA AVPRPQPRALGRADAELAAGGDAHQRAAGLPLLPPRLPRRGGVQLSG	SEQ ID NO 5 and 6
>CDS3448 BAA89798.1 OsNAC4 (rice) MAAAVGGSGRRDAEAELNLPPGFRFHPTDEELVVHYLCRKVARQPLP VPIIAEVDLYKLDPWDLPEKALFGRKEWYFFTPRDRKYPNGSRPNRAA GRGYWKATGADKPVAPKGSARTVGIKKALVFYSGKAPRGVKTDWIMH EYRLADADRAPGGKKGSQKLDEWVLCRLYNKKNNWEKVKLEQQDVA SVAAAAPRNHHHQNGEVMDAAAADTMSDSFQTHDSDIDNASAGLRHG GCGGGGFGDVAPPRNGFVTVKEDNDWFTGLNFDELQPPYMMMNLQHM QMQMVNPAAPGHDGGYLQSISSPQMKMWQTILPPF	SEQ ID NO 7 and 8
>CDS3447 AAD37699.1 OS Homeodomain leucine zipper protein HOX6 (rice) MDGEEDSEWMMMDVGGKGGKGGGGGGAADRKKRFSEEQIKSLESM	SEQ ID NO 9 and 10

FATQTKLEPRQKLQLARELGLQPRQVAIWFQNKRARWKSKQLEREYSA LRDDYDALLCSYESLKKEKLALIKQLEKLAEMLQEPRGKYGDNAGDDA RSGGVAGMKKEEFVGAGGAATLYSSAEGGGTSSTEQTCSSTPWWEF ESE	
>CDS3446 AK104073 OSMYB predicted (rice) MASIVTATVAAASAWWATQGLLPLFPPPIAFPFVPAPSAPFSTADVQRA QEKDIDCPMDNAQKELQETRKQDNFEAMKVIVSSETDESGKGEVSLHT ELKISPADKADTKPAAGAETSDVFGNKKKQDRSSCGSNTPSSSDIEAD NAPENQEKANDKAKQASCSNSSAGDNNHRRFRSSASTSDSWKEVSE EGRLAFDALFSRERLPQSFSPPQVEGSKEISKEEEDEVTTVTVDLNKNA AIIDQELDTADEPRASFPNELSNLKLKSRRTGFKPYKRCSVEAKENRVP ASDEVGTKRIRLESEAST	SEQ ID NO 11 and 12
>CDS3445 NP_565887.1 At2g38310 (*Arabidopsis*) MLAVHRPSSAVSDGDSVQIPMMIASFQKRFPSLSRDSTAARFHTHEVG PNQCCSAVIQEISAPISTVWSVVRRFDNPQAYKHFLKSCSVIGGDGDNV GSLRQVHVVSGLPAASSTERLDILDDERHVISFSVVGGDHRLSNYRSVT TLHPSPISGTVVVESYVVDVPPGNTKEETCDFVDVIVRCNLQSLAKIAEN TAAESKKKMSL	SEQ ID NO 13 and 14
>CDS3444 NP_565703.1 At2g30590WRKY family transcription factor (*Arabidopsis*) MEEIEGTNRAAVESCHRVLNLLHRSQQQDHVGFEKNLVSETREAVIRF KRVGSLLSSSVGHARFRRAKKLQSHVSQSLLLDPCQQRTTEVPSSSSQ KTPVLRSGFQELSLRQPSDSLTLGTRSFSLNSNAKAPLLQLNQQTMPP SNYPTLFPVQQQQQQQQQQQQQEQQQQQQQQQQQFHERLQAHHL HQQQQLQKHQAELMLRKCNGGISLSFDNSSCTPTMSSTRSFVSSLSID GSVANIEGKNSFHFGVPSSTDQNSLHSKRKCPLKGDEHGSLKCGSSSR CHCAKKRKHRVRRSIRVPAISNKVADIPPDDYSWRKYGQKPIKGSPYPR GYYKCSSMRGCPARKHVERCLEDPAMLIVTYEAEHNHPKLPSQAITT	SEQ ID NO 15 and 16
>CDS3443 NP_849867.1 At1g69510 (*Arabidopsis*) MEDVKGKEIIDDAPIDNKVSDEMESEENAIKKKYGGLLPKKIPLISKDHE RAFFDSADWALGKQKGQKPKGPLEALRPKLQPTPQQQPRARRMAYSS GETEDTEIDNNEAPDDQACASAVDSTNLKDDGGAKDNIKS	SEQ ID NO 17 and 18
>CDS3442 NP_564615.3 At1g52870 (*Arabidopsis*) MAAASLHTSISPRSFLPLSKPSLKPHRSQILLRNKQRNCVSCALIRDEID LIPVQSRDRTDHEEGSVVVMSTETAVDGNESVVVGFSAATSEGQLSLE GFPSSSSSGADLGDEKRRENEEMEKMIDRTINATIVLAAGSYAITKLLTI DHDYWHGWTLFEILRYAPQHNWIAYEEALKQNPVLAKMVISGVVYSVG	SEQ ID NO 19 and 20

DWIAQCYEGKPLFEIDRARTLRSGLVGFTLHGSLSHFYYQFCEELFPFQ DWWVVPVKVAFDQTVWSAIWNSIYFTVLGFLRFESPISIFKELKATFLPM LTAGWKLWPFAHLITYGLVPVEQRLLWVDCVELIWVTILSTYSNEKSEA RISESVIETSSSSTTTIDPSKE	
>CDS3441 NP_849293.1 At4g02920 (*Arabidopsis*) MIKLCFMTSHGYSIPGLGLPQDLCNTEIIKQNSRSHLVNPGARQEIIPAS SFNLNTELLEPWKPVSSFSQFVEIDSAMMKPLLMDVHETAPESLILSFGI ADKFARQEKVMEFLLSQSEEFKEKGFDMSLLNELMEFESMKSSSQLRP YDTSSVLYLNQELGKPVLDLVRDMMENPEFSVRSNGHVLFSSSSNPEL NDLLSIASEFNLSRNSTTKWRQLSPLIPHFQRFESDVFTPAKLKAVTVLA PLKSPEKSRLKSPRKHNTKRKAKERDLYKRNHLHAYESLLSLMIGNDH RHKHTTVLSLQKSCGELSELLTQFSITAAGTGIAVLFSVVCSLASRRVPF CANKFFDTGLGLSLVILSWAVNRLREVIVHVNRKANKPCSSLKDDEIINS VERSMKEVYYRAATVIAVFALRFAC	SEQ ID NO 21 and 22
>CDS3440 AAM91100.1 At1g45200 (*Arabidopsis*) MSKTNMKFCNSYFLVDPTKASFLDLLLLLFSSNLTSARFIDSPPDTLKGF RRSFASRWILALAIFLQKVLMLLSKPFAFIGQKLTYWLNLLTANGGFFNLI LNLMSGKLVKPDKSSATYTSFIGCSDRRIELDEKINVGSIEYKSMLSIMA SKISYESKPYITSVVKNTWKMDLVGNYDFYNAFQESKLTQAFVFKTSST NPDLIVVSFRGTEPFEAADWCTDLDLSWYEMKNVGKVHAGFSRALGL QKDGWPKENISLLHQYAYYTIRQMLRDKLGRNKNLKYILTGHSLGGALA ALFPAILAIHGEDELLDKLEGIYTFGQPRVGDEDFGEFMKGVVKKHGIEY ERFVYNNDVVPRVPFDDKYLFSYKHYGPCNSFNSLYKGKVREDAPNA NYFNLLWLIPQLLTGLWEFIRSFILQFWKGDEYKENWLMRFVRVVGIVF PGGSNHFPFDYVNSTRLGGLVRPPPTTTPEDKLALIA	SEQ ID NO 23 and 24

Transgenic plants generated by these rice transformation methods are evaluated for various growth characteristics. More particularly, the transgenic plants are evaluated and the following parameters are monitored: increased total above ground biomass, increased plant height, increased number of tillers, increased number of first panicles, increased number of second panicles, increased total number of seeds, increased number of filled seeds, increased total seed yield (weigth) per plant, increased harvest index, increased thousand kernel weight, increased Tmid, increased T45 or A90, increased A42, changed cycling time or an changed growth curve, changed flowering time.

Plants with increase biomass, increased organ number and/or size (including seeds) and or any other economically attractive growth characteristics as found by the following plant

evaluation protocol, are selected to transferring the transgenic traits into commercial germplasm.

Evaluation protocol for T0, T1 and T2 transgenic rice plants transformed with an E2F target gene according to the present invention

Approximately 15 to 20 independent T0 rice transformants are generated. The primary transformants are transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seed. Approximately 6 events, of which the T1 progeny segregated 3:1 for presence/absence of the transgene, are retained. For each of these events, approximately 10 T1 seedlings containing the transgene (hetero- and homo-zygotes), and approximately 10 T1 seedlings lacking the transgene (nullizygotes), are selected by monitoring screenable marker expression.

2 events with improved agronomical parameters in T1 are chosen for re-evaluation in T2 generation. Seed batches from the positive plants (both hetero- and homozygotes) in T1, are screened by monitoring marker expression. For each chosen event, the heterozygote seed batches are then selected for T2 evaluation. An equal number of positives and negatives within each seed batch are transplanted for evaluation in the greenhouse. The total number of 120 transformed plants is evaluated in the T2 generation. More particularly, two transformed events are selected, 60 plants per event of which 30 positives for the transgene, and 30 negative. T1 and T2 plants are transferred to the greenhouse and evaluated for vegetative growth parameters and seed parameters, as described hereunder.

Statistical analysis: t-test and F-test

A two factor ANOVA (analysis of variants) is used as statistical model for the overall evaluation of plant phenotypic characteristics. An F-test is carried out on all the parameters measured, for all of the plants of all of the events transformed with the gene of interest. The F-test is carried out to check for an effect of the gene over all the transformation events and to determine the overall effect of the gene or "global gene effect". Significant data, as determined by the value of the F-test, indicates a "gene" effect, meaning that the phenotype observed is caused by more than the presence or position of the gene. In the case of the F-test, the threshold for significance for a global gene effect is set at a 5% probability level.

To check for an effect of the gene within an event, i.e., for a line-specific effect, a t-test is performed within each event using data sets from the transgenic plants and the corresponding null plants. "Null plants" or "Null segregants" are the plants treated in the same way as the transgenic plant, but from which the transgene has segregated. Null plants can also be described as the homozygous negative transformants. The threshold for significance for the

t-test is set at 10% probability level. Within one population of transformation events, some events can be under or above this t-test threshold. This is based on the hypothesis that a gene might only have an effect in certain positions in the genome, and that the occurrence of this position-dependent effect is not uncommon. This kind of gene effect may also be referred to as a "line effect of a gene". The p-value is obtained by comparing the t-value to the t-distribution or alternatively, by comparing the F-value to the F-distribution. The p-value stands for the probability that the null hypothesis (null hypothesis being "there is no effect of the transgene") is correct.

Vegetative growth measurements:

The selected transgenic plants are grown in a greenhouse. Each plant receives a unique barcode label to link unambiguously the phenotyping data to the corresponding plant. The selected transgenic plants are grown on soil in 10 cm diameter pots under the following environmental settings: photoperiod= 11.5 h, daylight intensity= 30,000 lux or more, daytime temperature= 28°C or higher, night time temperature= 22°C, relative humidity= 60-70%. Transgenic plants and the corresponding nullizygotes were grown side-by-side at random positions. From the stage of sowing until the stage of maturity each plant is passed several times through a digital imaging cabinet and imaged. At each time point digital images (2048x1536 pixels, 16 million colours) are taken of each plant from at least 6 different angles. The parameters described below are derived in an automated way from all the digital images of all the plants, using image analysis software.

(a) Above ground plant area is determined by counting the total number of pixels from aboveground plant parts discriminated from the background. This value is averaged for the pictures taken on the same time point from the different angles and converted to a physical surface value expressed in square mm by calibration. Experiments show that the aboveground plant area measured this way correlates with the biomass of plant parts above ground.

(b) Plant height is determined by the distance between the horizontal lines going through the upper pot edge and the uppermost pixel corresponding to a plant part above ground. This value is averaged for the pictures taken on the same time point from the different angles and was converted, by calibration, to a physical distance expressed in mm. Experiments showed that plant height measured this way correlate with plant height measured manually with a ruler.

(C) Number of primary tillers is manually counted at the harvesting of the plants. The tillers are cut off at 3 cm above the pot rim. They were then counted at the cut surface. Tillers that were together in the same sheet were counted as one tiller.

(d) Number of primary panicles. The tallest panicle and all the panicles that overlap with the tallest panicles when aligned vertically are counted manually, and considered as primary panicles.

(e) Number of secondary panicles. The number of panicles that remained on the plant after the harvest of the primary panicles are counted and considered as secondary panicles.

(f) Growth curve. The plant area weekly measurements are modeled to obtain a growth curve for each plant, plotted as the value of plant area (in mm^2) over the time (in days). From this growth curve the following parameters are calculated.

(g) A42 is the plant area at day 42 after sowing as predicted by the growth curve model.

(h) Tmid is the time that a plant needs to grow and to reach 50% of the maximum plant area. Tmid is predicted from the growth curve model.

(i) T90 is the time that a plant needs to grow and to reach 90% of the maximum plant area. T90 is predicted from the growth curve model.

Seed-related parameter measurements

The mature primary panicles of T1 and T2 plants are harvested, bagged, barcode-labelled and then dried for three days in the oven at 37°C. The panicles are then threshed and all the seeds were collected and counted. The filled husks are separated from the empty ones using an air-blowing device. The empty husks are discarded and the remaining fraction is counted again. The filled husks are weighed on an analytical balance. This procedure resulted in the set of seed-related parameters described below.

(a) Total seed number per plant is measured by counting the number of husks harvested from a plant.

(b) Number of filled seeds is determined by counting the number of filled husks that remained after the separation step.

(c) Total seed yield per plant is measured by weighing all filled husks harvested from a plant.

(d) Harvest index of plants is defined as the ratio between the total seed yield and the above ground area (mm^2), multiplied by a factor 10^6.

(e) Thousand Kernel Weight (TKW) of plants is a parameter extrapolated from the number of filled seeds counted, and their total weight.

(f) TotalArea Emergence Prop. is the time when plant reach 30 % of their maximum total area

(g) TotalArea Cycle Time. *i*s the time when plant reach 90 % of their maximum total area

Further molecular analysis is performed on the positive plants by techniques well known by the person skilled in the art such as for example RT-PCR.

Tables

Table 1. Arabidopsis Genes 2-fold or more upregulated in E2Fa/DPa plants

Gene Identification	accession #	MIPS name	OLD REF		SEQ ID NO		Fold induction	E2F site	Plant homologue
			cDNA	PROT	cDNA	PROT			
Unknown function (14)									
hypothetical protein	AI998042	At1g57680	1	53	433	434	2.66		rice BAB90159.1, maize AY107220.1
putative protein	AI994686	At3g45730	2	54	231	232	5.14		
putative protein	AI994734	At5g66580	4	56	489	490	3.18		
unknown protein	AI999397	At2g38310	5	57	995	996	2.79	TTTGCCC C	rice BAB68102.1
unknown protein	AI995465	At2g47440	7	59	931	932	2.50		
unknown protein	AI994871	At1g76970	8	60	1193	1194	2.34		rice BAB78689.1, corn AAB00079.1
hypothetical protein, kinesin	AI998366	At1g27500	9	61	393	394	2.21		rice AAL87057.1
putative protein	AI996967	At4g33050	10	62	883	884	2.20		rice BAB90008.1
putative protein	AI995917	At3g43690	12	64	263	264	2.18		
unknown protein, kh domain protein	AI993084	At2g25970	13	65	941	942	2.15		rice BAA92910.1, maize AY106526.1
unknown protein	AI993077	At1g68580	14	66	937	938	2.13		rice BAC00723.1, corn AAK11516.1
putative protein, copine	AI993019	At5g14420	15	67	205	206	2.05		rice BAB92575.1
hypothetical protein	AI997428	At1g57990	16	68	415	416	2.02		rice BAB90042.1
unknown protein	AI997827	At5g53740	17	69	2731	2732	2.01		
DNA replication and modification (14)									
putative thymidine kinase	AI997851	At3g07800					8.44		rice AAC31168.1
DNA methyltransferase	AI994691	At5g49160					5.37	ATTGCCG C	rice AAL77415.1, corn AAC16389.1
Msi3	AW004204	At4g35050					4.89	TTTCCCG C	corn AAL33648.1
putative linker histone protein	AI994590	At3g18035					3.31		
putative replication factor c	AI997934	At1g21690					3.30	TTTCCCG C	
topoisomerase 6 subunit A	AI995290	At5g02820					2.62	TTTCCCG C	
histone H4-like protein	AI999171	At3g46320					2.55	TTTGGCG C	
histone acetylase HAT B	AI998229	At5g56740					2.36	TTTCCCG C	corn AAM28228.1
putative histon H1	AI996137	At1g06760					2.27		
histone H2A-like protein	AI995882	At4g27230					2.23		
putative DNA gyrase subunit A	AI995400	At3g10690					2.20		rice AAD29710.1
histone H2B-like protein	AI999101	At5g59910					2.16		
putative mismatch binding protein	AI993280	At3g24320					2.10		rice CAD41187.1, corn AAF35250.1
adenosylhomocysteinase	AI996953	At4g13940					2.07		corn AAL33588.1

Cell Cycle (2)									
E2Fa	AJ294534	At2g36010					94.88		
CDKB1;1	D10851	At3g54180					2.60	TTTCCCGC	
Cell wall biogenesis (11)									
xyloglucan endo-1;4-beta-D-glucanase (meri-5)	AI994459	At4g30270					3.74		
putative glycosyl transferase	AI999244	At1g70090					3.38		
alpha galactosyltransferase-like protein	AI998223	At3g62720					3.26		
putative xyloglucan endotransglycosylase	AI999683	At3g23730					2.85		rice CAD41426.1, corn CAB510059.1
xyloglucan endo-1,4-beta-D-glucanase-like protein	AI998301	At4g30280					2.74		
putative xyloglucan endotransglycosylase	AI994477	At1g14720					2.51		
putative glycosyl transferase	AI999770	At1g24170					2.39		
putative UDP-glucose glucosyltransferase	AI997288	At1g22400					2.34	TTTCCCGC	
putative glucosyltransferase	AI998872	At2g15480					2.15		
peroxidase	AI994622	At2g38380					2.11	TTTCGCCC	
beta-1,3-glucanase-like protein	AI994681	At3g55430					2.05		rice AAB37697.1, corn CAB96424.1
Chloroplastic genes (7)									
large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase	N96785	rbcL			2713	2714	4.71		NP_051067
ribosomal protein L33	AI994194	rpl33			2715	2716	3.54		NP_051080
PSII I protein	AW004203	psbI			2717	2718	2.81		NP_051074
ribosomal protein L2	AW004266	rpl2			2719	2720	2.61		NP_051099
ATP-dependent protease subunit	AI997947	clpP			2721	2722	2.60		NP_051083
cytochrome B6	AI997102	petB			2723	2724	2.55		NP_051088
ATPase epsilon subunit	AW004251	atpE			2725	2726	2.17		NP_051065
Mitochondrial genes (1)									
26S ribosomal RNA protein	AW004275	orf107a			2727	2728	2.87		NP_085475
Transcription factors (6)									
LOB domain protien 41	AI996685	At3g02550	3	55	1109	1110	4.01		riceBAB92193.1
WRKY transcription factor 21	AI992739	At2g30590					2.78	TTTCCCCC	
GATA Zn-finger protein	AI995731	At3g16870	6	58	2729	2730	2.75		maize AY072149
Anthocyaninless2	AI993655	At4g00730					2.73	TTTCCCCC	
leucine zipper-	AI995691	At1g07000					2.43		

containing protein									
homeodomain transcription factor (Athb-6)	AI999190	At2g22430					2.30		rice CAA65456.2, corn CAB96424.1
Metabolism and biogenesis (11)									
alcohol dehydrogenase	AI998773	At1g77120					5.09		
putative isocistrate lyase	AI999168	At3g21720					3.08		
protochlorophyllide reductase precursor	AI993342	At4g27440					2.39		
suger transpoter like protein	AI997793	At4g36670					2.27		rice AAK13147.1, corn AAF74568.1
NADH-dependent glutamate synthase (GOGAT)	AI997600	At5g53460					2.25		
nitrate reductase (NIA2)	AI996208	At1g37130					2.15		
pectate lyase - like protein	AJ508995	At3g54920					2.13		
putative sterol dehydrogenase	AI996340	At2g43420					2.10		
glutamine synthetase root isozyme 1 (GS)	161G19T7	At1g66200					2.06		
monosaccharide transporter STP3	AI997045	At5g61520					2.05		rice BAA83554.1, corn AAF74568.1
Signal transduction (6)									
calcium-dependent protein kinase	AI996555	At5g66210					2.96		rice AAF23901.2, corn BAA12715.1
WD-40 repeat protein	AI993055	At5g14530					2.70		rice AD27557.1, corn AAA50446.1
receptor-protien kinase-like protein	AI994727	At5g54380					2.59		rice AAK63934.1, corn AAB09771.1
putative phytochrome A	AI998146	At1g09570					2.45		
putative leucine-rich receptor-like protein kinase	AI999651	At1g72180					2.13		rice BAC06203.1, corn CAC35411.1
putative receptor-like kinase	AI993298	At3g23750					2.06		rice CAA69028.1, corn CAC35412.1
Others (13)									
putative pollen allergen	AI996548	At3g45970					3.22		rice AAG13596.1, corn CAD40849.1
cold-regulated protein COR6,6	AW004198	At5g15970					3.03		
phi-1-like protein	AI994601	At5g64260					2.60		
lipid-transfer protein-like	AI998609	At5g01870					2.33		rice BAB86497.1, corn AAB06443.1
DnaJ homologue	AI994551	At5g06910					2.32	ATTGGCGC	
blue copper binding protein	AI996535	At5g20230					2.30		
src-2 like protein	AI998679	At1g09070	11	63	401	402	2.19		
RING finger protein	AI999491	At3g61460					2.14		rice BAA85438.1, corn AAL59234.1
putative Ticc22	AI993361	At3g23710					2.14		
nodulin-like protein	AI996322	At1g80530					2.07		rice AAM01022.1
putative resistance protein	AI997549	At1g61100					2.06		rice AAL83695.1,

seed imbitition protein-like	AI993446	At5g20250					2.05		
putative disease resistance protein	AI998978	At1g72900					2.04		rice AAL01163.1, corn AAC83564.1

Table 2. Arabidopsis Genes 2-fold or more repressed in E2Fa/DPa plants

Gene Identification	accession #	MIPS	OLD REF		SEQ ID NO		Fold repression	E2F site	plant hom ologue
			cDNA	PROT	cDNA	PROT			
Unknown function (35)									
unknown protein	AI993767	At1g45200*	18	70	2741	2742	3.91		
putative protein	AI993468	At3g56290	19	71	1483	1484	3.38		maize AY106321.1 , rice BAB93184.1
hypothetical protein, multidrug efflux protein	AI996374	At1g61890	21	73	2599	2600	2.78		
unknown protein	AI994573	At3g15950	22	74	2147	2148	2.71		
putative protein	AI994726	At3g52360	23	75	1619	1620	2.65		
hypothetical protein	AI997393	At4g02920	24	76	1521	1522	2.60	TTTGCCCC	Y09602. Hordeum vulgare
unknown protein, put protease inhibitor	AJ508997	At5g43580	25	77	2743	2744	2.58		
unknown protein	AI997866	At1g70760	26	78	2077	2078	2.52		
unknown protein	AI997085	At5g43750	27	79	1423	1424	2.51		rice BAB90754.1
putative protein	AI995724	At5g50100**	28	80	1973	1974	2.48		rice AL606619.2 OSJN00032 genomic
unknown protein	AI995337	At1g74880	29	81	2699	2700	2.42		maize AY105515.1, rice BAB89011.1
unknown protein	AI998296	At3g19370	30	82	1859	1860	2.40		
unknown protein, ATP ase	AI993346	At3g10420	31	83	2249	2250	2.40		
putative protein	AI999485	At3g61080	32	84	1863	1864	2.38		
unknown protein	AI996923	At1g67860	33	85	1847	1848	2.38		
unknown protein	AI994841	At1g52870	34	86	2367	2638	2.35	ATTCCCCC	maize AY108423.1
unknown protein	AI999581	At1g64370	35	87	2099	2100	2.35		
unknown protein	AI997584	At1g05870	36	88	1955	1956	2.25		rice BAB86085.1, maize Y110580.1
putative protein	AI992938	At5g03540	37	89	2745	2746	2.21		
hypothetical protein	AI997712	At2g15020	38	90	2605	2606	2.21		rice BAB64794.1
unknown protein	AI998338	At1g68440	39	91	2625	2626	2.20		
unknown protein	AI996872	At2g21960	40	92	1715	1716	2.19		
putative protein, centrin	AI996295	At4g27280	41	93	2039	2040	2.18		
putative protein	AI995642	At3g48200	42	94	2653	2654	2.16		
unknown protein	AI997470	At2g32870	43	95	1941	1942	2.14		
hypothetical protein	AI998460	At1g69510	44	96	2019	2020	2.11	TTTGGCCC	rice BAB18340.1, maize AY110240.1
putative triacylglycerol lipase	AI993356	At5g22460	45	97	2349	2350	2.10		
putative protein	AI995956	At5g52060	46	98	1779	1780	2.08		
unknown protein	AI996100	At2g35830	47	99	2471	2472	2.06		
hypothetical protein	AI996039	At3g27050	48	100	2175	2176	2.05		
unknown protein	AI996020	At5g51720	49	101	2033	2034	2.04		
putative protein	AW004101	At4g39730	51	103	1605	1606	2.03		
hypothetical protein	AI998372	At2g01260	52	104	1979	1980	2.03		

unknown protein	AI999573	At3g61060					2.00		
unknown protein	AI998562	At2g35760					2.00		
No hit (2)									
no hit on genome	AI995690						2.54		
no hit on genome	AI999010						2.23		
Cell wall biogenesis (4)									
similar to polygalacturonase-like protein	AI993509	At1g10640	50	102	1761	1762	3.62		maize AY106712.1, rice BAC06884.1
putative xyloglucan endo-transglycosylase	AI997647	At2g36870					2.51		
pectate lyase 1-like protein	AI994801	At1g67750					2.40		
xyloglucan endo-transglycosylase	AI998832	At3g44990					2.35		
Metabolism and biogenesis (24)									
fructose-biphosphate aldolase-like protein	AI994456	At4g26530					5.99	ATTGGCCC	
sucrose-phosphate synthase-like protein	AI995432	At4g10120					4.64		
putative branched-chain amino acid aminotransferase	AI997263	At3g19710					3.31		
vitamine c-2	AI997404	At4g26850	20	72	2511	2512	3.04	TTTGCCGC	maize AY105327, rice BAB90526.1
nicotianamine synthase	AI993200	At5g04950					2.86		
beta-fructosidase	AI994670	At1g62660					2.66	TTTCCCCC	
neoxanthin cleavage enzyme-like protein	AI997269	At4g19170					2.66		
putative starch synthase	AI997174	At1g32900					2.63		
cytochrome P450 monooxygenase (CYP83A1)	AI994017	At4g13770					2.57		
beta-amylase-like protein	AI999322	At5g18670					2.53		
FRO1-like protein; NADPH oxidase-like	AI995987	At5g49740					2.46		
putative hydrolase	AI997149	At3g48420					2.39		
furamate hydratase	AI997067	At5g50950					2.31	TTTGGCCC	
5'-adenylylsulfate reductase	AI992757	At1g62180					2.30	TTTCCCCC	
5´-adenylylsulfate reductase	AI996614	At4g04610					2.30		
UDP rhamnose-anthocyanidin-3-glucoside rhamnosyltransferase -like protein	AI996803	At4g27560					2.24		
cytochrome P450-like protein	AI993171	At5g48000					2.23		
lactoylglutathione lyase-like protein	AI994552	At1g11840					2.20		
putative beta-glucosidase	AI995306	At4g27820					2.20	ATTGGCCC	

adenine phospho-ribosyltransferase-like protein	AI994567	At4g22570					2.18		
catalase	AI995830	At4g35090					2.17	ATTCCCCC	
putative glutathione peroxidase	AW004143	At2g25080					2.15		
putative adenosine phosphosulfate kinase	AW004219	At2g14750					2.13		
tyrosine transaminase like protein	AI996914	At4g23600					2.13		
Transcription factors (5)									
homeobox-leucine zipper protein ATHB-12	AI994027	At3g61890					4.20		
NAC domain protein NAC2	AI992865	At1g69490					3.68		
myb-related transcription factor	AI995298	At1g71030					2.78		
dof zinc finger protein	AI994875	At1g51700					2.30		
MYB-related transcription factor (CCA1)	AI992931	At2g46830					2.19		
Signal transduction (9)									
serine/threonine protein kinase-like protein	AI995557	At5g10930					3.91		
subtilisin proteinase-like	AI993428	At4g21650					3.19		
putative oligopeptide transporter	AI996160	At4g10770					2.68		
putative lectin	AI998542	At3g16400					2.52		
Ca2+dependent membrane-binding protein annexin	AI998553	At1g35720					2.45		
putative WD repeat protein	AI997238	At3g15880					2.38		
putative lectin	AI999016	At3g16390					2.35		
putative lectin	AI993358	At3g16530					2.31		
SNF1 related protein kinase (ATSRPK1)	AI993111	At3g23000					2.06		
Others (25)									
putative protease inhibitor Dr4	AI995265	At1g73330					10.30		
major latex protein homolog - like	AI998305	At2g01520					4.27		
pollen allergen-like protein	AI993041	At1g24020					3.56		
putative heat shock protein	AI997846	At1g06460					3.55		
putative fibrillin	AI997199	At4g04020					3.55		
major latex protein homolog - like	AI997255	At1g70890					3.50		
putative nematode-resistance protein	AI993740	At2g40000					2.95		
putative auxin-regulated protein	AJ508998	At2g46690					2.86		
putative myrosinase-binding protein	AI997583	At2g39310					2.61		
ubiquitin-conjugating enzyme-like protein	AI997782	At5g56150					2.41		

ubiquitin-conjugating enzyme E2-17 kD 8	AI994771	At5g41700					2.40		
vegetative storage protein Vsp2	AI999152	At5g24770					2.35		
heat shock protein 70	AI994044	At3g12580					2.24		
chloroplast outer envelope membrane protein	AI997015	At3g63160					2.20		
translation initiation factor-like protein	AI992786	At5g54940					2.15		
pseudogene	AI995323	At2g04110					2.07		
vegetative storage protein Vsp1	AI999546	At5g24780					2.06		
dehydrin ERD10	AI997518	At1g20450					2.06		
MTN3-like protein	AI997159	At3g48740					2.05		
putative chlorophyll A-B binding protein	AI994859	At3g27690					2.05		
photosystem I reaction centre subunit psaN	AI997939	At5g64040					2.03		
AR781, similar to yeast pheromone receptor	AI998194	At2g26530					2.03		
putative lipid transfer protein	AI997024	At2g15050					2.03		
peroxidase ATP3a	AI998372	At5g64100					2.03		
myosin heavy chain-like protein	AI999224	At3g16000					2.01		

- * this sequence is present in the MIPs database version of 25 july 2002
- ** this record has an updated MIPS accession number At5g50101.

Table 3. Number of E2F elements in the different datasets

	All genes (4518)	Upregulated genes (88)	Downregulated genes (105)
TTTCCCCC	62	2	3
TTTCCCGC	40	6	0
TTTCGCCC	15	0	0
TTTCGCCC	13	1	0
TTTGCCCC	37	1	1
TTTGCCGC	20	0	1
TTTGGCCC	55	0	2
TTTGGCGC	15	1	0
ATTCCCCC	10	0	2
ATTCCCGC	6	0	0
ATTCGCCC	8	0	0
ATTCGCCC	14	0	0
ATTGCCCC	13	0	0
ATTGCCGC	10	1	0
ATTGGCCC	44	0	2
ATTGGCGC	9	1	0
Total	371	13	11

Table 4: Arabidopsis genes 1.3 fold or more upregulated in E2Fa/Dpa plants

SEQ ID NO		Gene name	e_value	MIPS accession number	ratio
cDNA	PROT				

25	26	putative protein	0	At5g51100	1.42
27	28	endo-1,4-beta-glucanase	9E-27	At1g70710	1.85
29	30	mitochondrial elongation factor Tu	1E-125	At4g02930	1.39
31	32	glycine-rich protein (clone AtGRP8)	1E-155	At4g39260	1.33
33	34	UTP-glucose glucosyltransferase	0	At5g66690	1.59
35	36	lipid-transfer protein-like	0	At5g01870	2.33
37	38	putative auxin-regulated protein	6E-68	At4g34760	1.48
39	40	histone H1, putative	0	At1g06760	2.27
41	42	APETALA2 protein	0	At4g36920	1.44
43	44	putative histone H2A	0	At1g08880	1.84
45	46	monosaccharide transporter STP3	2E-69	At5g61520	2.05
47	48	receptor-protein kinase-like protein	8E-64	At3g51550	1.33
49	50	SET-domain protein-like	1E-140	At5g04940	1.38
51	52	homeodomain transcription factor (ATHB-6)	0	At2g22430	2.3
53	54	putative protein	0	At4g33700	1.85
55	56	hypothetical protein	1E-139	At1g05800	1.34
57	58	unknown protein	0	At1g33410	1.37
59	60	hypothetical protein	1E-140	At4g17060	1.41
61	62	putative protein	0	At5g19820	1.44
63	64	putative protein	1E+00	At3g53670	1.54
65	66	regulatory subunit of protein kinase CK2	0	At3g60250	1.51
67	68	delta 9 desaturase, putative	0	At1g06090	1.85
69	70	putative protein	0	At5g06360	1.48
71	72	acetyl-CoA carboxylase, putative, 5' partial	0	At1g36170***	1.49
73	74	hypothetical protein	0	At1g56150	1.97
75	76	seed imbitition protein-like	0	At5g20250	2.05
77	78	unknown protein	1E-146	At1g76010	1.64
79	80	homeobox-leucine zipper protein-like	0	At5g47370	2.21
81	82	kinesin-like protein	0	At5g54670	1.69
83	84	putative protein	0	At3g48050	1.75
85	86	putative protein	0	At5g03040	1.34
87	88	xyloglucan endo-1,4-beta-D-glucanase precursor	0	At4g30270	3.74
89	90	putative WD-40 repeat protein	0	At2g19540	1.75
91	92	putative protein	1E-132	At3g54480	1.44
93	94	hypothetical protein	0	At1g15750	1.7
95	96	hypothetical protein	0	At1g66200	2.06
97	98	putative protein	0	At3g50630	1.4
99	100	unknown protein	0	At2g30930	1.3
101	102	putative protein	6E-91	At5g37720	1.8
103	104	unknown protein	1E-146	At5g54310	1.61
105	106	hypothetical protein	0	At1g48920	1.98
107	108	hypothetical protein	0	At1g17750	1.38
109	110	nuclear RNA binding protein A-like protein	0	At4g17520	1.43
111	112	unknown protein	4E+00	At1g10890	1.38
113	114	histone H2A- like protein	0	At4g27230	2.23
115	116	phytochelatin synthase (gb|AAD41794.1)	0	At5g44070	1.39
117	118	RNA-binding protein cp29 protein	1E-159	At3g53460	1.54
119	120	putative RNA-binding protein	0	At3g25150	1.48
121	122	alcohol dehydrogenase	2E-01	At5g42250	1.34
123	124	putative 60S ribosomal protein L6	1E-170	At1g74060	1.37
125	126	calmodulin-binding protein	1E-114	At5g57580	1.4
127	128	putative protein	3E-23	At4g20310	2.01

129	130	putative protein kinase	0	At1g08720	1.33
131	132	hypothetical protein	0	At3g12200	1.34
133	134	putative phosphatidylserine decarboxylase	0	At4g25970	1.38
135	136	unknown protein	0	At2g03120	1.31
137	138	unknown protein	0	At1g14880	1.48
139	140	histone H2A.F/Z	0	At3g54560	1.85
141	142	4-coumarate-CoA ligase - like	0	At4g19010	1.35
143	144	putative protein	0	At3g45040	1.72
145	146	unknown protein	0	At3g19540	1.84
147	148	putative protein	0	At4g34410	1.36
149	150	unknown protein	0	At1g61260	1.97
151	152	putative protein	0	At3g61490	1.32
153	154	lipoxygenase	0	At1g17420	1.34
155	156	putative SecA-type chloroplast protein transport factor	0	At4g01800	1.38
157	158	putative DNA-binding protein	0	At4g01250	1.49
159	160	hypothetical protein	0	At1g20580	1.37
161	162	hypothetical protein	2E-90	At1g47530	1.39
163	164	unknown protein	0	At2g37570	1.84
165	166	bZIP transcription factor-like protein	0	At3g62420	1.32
167	168	putative protein	1E-154	At3g56720	1.39
169	170	hypothetical protein	0	At1g76860	1.32
171	172	6-phosphogluconate dehydrogenase	2E-80	At5g41670	1.48
173	174	ferritin 1 precursor	0	At5g01600	1.38
175	176	putative ABC transporter	0	At1g71330	1.71
177	178	hypothetical protein	0	At1g27300	1.3
179	180	myrosinase precursor	9E-01	At5g26000	2.81
181	182	unknown protein	0E+00	At1g10270	1.47
183	184	putative protein	3E-88	At5g18650	1.33
185	186	hypothetical protein	6E-40	At2g36090	1.32
187	188	unknown protein	0	At1g43910	1.42
189	190	hypothetical protein	0	At1g07000	2.43
191	192	hypothetical protein	0	At1g18260	1.43
193	194	putative pre-mRNA splicing factor	0	At4g03430	1.49
195	196	putative protein	0	At5g11810	1.32
197	198	hypothetical protein	1E-151	At4g30150	1.41
199	200	S-receptor kinase -like protein	0	At4g32300	1.52
201	202	disease resistance RPP5 like protein	1E-175	At4g16950	1.64
203	204	unknown protein	2E-58	At1g76520	1.44
205	206	putative protein	1E-144	At5g14420	2.05
207	208	putative glucosyltransferase	4E-78	At1g23480	1.31
209	210	putative protein	1E-144	At4g28470	1.34
211	212	putative protein	0	At4g29830	1.55
213	214	putative auxin-regulated protein	0	At2g33830	1.41
215	216	putative protein	8E+00	At5g61550	1.38
217	218	unknown protein	0	At1g44810	1.39
219	220	protein phosphatase - like protein	1E-59	At5g02760	1.76
221	222	hypothetical protein	2E-21	At4g17800	1.59
223	224	hypothetical protein	0	At1g54080	1.58
225	226	xyloglucan endo-transglycosylase, putative	0	At1g14720	2.51
227	228	putative protein	0	At3g49320	1.7
229	230	beta-1,3-glucanase - like protein	0	At3g55430	2.05
231	232	putative protein	0	At3g45730	5.14

233	234	ubiquitin-conjugating enzyme E2-21 kD 1 (ubiquitin-protein ligase)	0	At5g41340	1.32
235	236	putative reticuline oxidase-like protein	0	At1g30720	1.31
237	238	DNA (cytosine-5)-methyltransferase (DNA methyltransferase) (DNA	0	At5g49160	5.37
239	240	putative protein	0	At4g32030	1.38
241	242	unknown protein	3E+00	At2g32710	1.46
243	244	E2F transcription factor-1 E2F1	1E-155	At5g22220	1.52
245	246	putative protein	0	At5g48820	1.8
247	248	putative E2F5 family transcription factor	1E-154	At2g36010	94.9
249	250	protein kinase cdc2 homolog B	0	At3g54180	2.6
251	252	putative WRKY DNA-binding protein	1E-164	At2g03340	1.43
253	254	hypothetical protein	0	At4g13670	1.56
255	256	xyloglucan endo-1,4-beta-D-glucanase-like protein	0	At4g30280	2.74
257	258	hypothetical protein	1E-121	At1g18630	1.41
259	260	putative protein	0	At5g35735	1.52
261	262	putative protein kinase	0	At2g47060	1.32
263	264	putative protein	1E-01	At3g43690	2.18
265	266	70kD heat shock protein	0	At2g32120	1.57
267	268	nitrate reductase	0	At1g37130	2.15
269	270	beta-amylase	0	At5g55700	1.55
271	272	multicatalytic endopeptidase complex alpha chain	0	At3g51260	1.57
273	274	putative protein	3E-02	At5g36190	2.55
275	276	putative protein	0	At4g00830	1.39
277	278	monodehydroascorbate reductase (NADH) - like protein	0	At5g03630	1.33
279	280	unknown protein	1E-107	At3g04350	1.42
281	282	hypothetical protein	0	At1g70090	3.38
283	284	E2 ubiquitin-conjugating-like enzyme Ahus5	0	At3g57870	1.38
285	286	putative protein	5E-25	At3g63070	1.35
287	288	hypothetical protein	0	At4g28330	2.23
289	290	cellulose synthase catalytic subunit, putative	1E-174	At1g55850	2.07
291	292	putative protein	0	At5g46410	1.54
293	294	putative polynucleotide phosphorylase	1E-136	At3g03710	1.53
295	296	hypothetical protein	0	At1g19180	1.32
297	298	hypothetical protein	0	At3g12270	1.83
299	300	sugar transporter like protein	0	At4g36670	2.27
301	302	hypothetical protein	1E-105	At2g39910	1.3
303	304	putative phytochrome A	0	At1g09570	2.45
305	306	hypothetical protein	0	At1g64600	1.49
307	308	putative protein	0	At5g23610	1.6
309	310	putative protein	1E-177	At3g56360	1.39
311	312	cyclophylin -like protein	0	At3g63400	1.33
313	314	unknown protein	0	At2g37940	1.35
315	316	zinc finger protein, putative	1E-53	At1g75540	1.46
317	318	putative protein kinase	1E+00	At2g24360	1.48
319	320	putative glucosyltransferase	0	At2g15490	2.15
321	322	unknown protein	0	At1g60140	1.72
323	324	unknown protein	0	At1g43850	1.45
325	326	hypothetical protein	0	At3g14120	1.77
327	328	putative AP2 domain transcription factor	0	At2g41710	1.65
329	330	transcriptional regulator protein, putative	6E-71	At3g26640	1.51
331	332	hypothetical protein	3E-02	At1g55370	1.35

333	334	unknown protein	0	At3g28920	1.93
335	336	hypothetical protein	0	At3g03750	1.43
337	338	hypothetical protein	2E+00	At4g27610	1.34
339	340	translation initiation factor eIF-2 beta chain - like protein	2E+00	At5g20920	1.33
341	342	unknown protein	0	At2g26280	1.53
343	344	unknown protein	0	At1g78420	1.39
345	346	elongation factor, putative	3E+00	At1g56070	1.99
347	348	anthranilate N-benzoyltransferase - like protein	1E-120	At5g01210	1.66
349	350	putative protein	1E-178	At4g39680	1.43
351	352	unknown protein	0	At3g05380	1.92
353	354	splicing factor At-SRp40	0	At4g25500	1.52
355	356	cdc2-like protein kinase	0	At5g10270	1.77
357	358	calcium-dependent protein kinase	1E-169	At3g57530	1.39
359	360	phosphoprotein phosphatase, type 1 catalytic subunit	0	At2g29400	1.48
361	362	putative mitochondrial translation elongation factor G	0	At2g45030	1.65
363	364	long-chain-fatty-acid--CoA ligase-like protein	0	At5g27600	1.34
365	366	cytochrome c, putative	4E-26	At3g27240	1.36
367	368	En/Spm-like transposon protein	0	At2g40070	1.41
369	370	putative phospho-ser/thr phosphatase	0	At4g03080	1.41
371	372	chloroplast 50S ribosomal protein L22, putative	6E-77	At1g52370	1.4
373	374	unknown protein	0	At2g15890	1.34
375	376	putative protein	0	At4g26750	1.55
377	378	receptor-protein kinase-like protein	0	At5g54380	2.59
379	380	phosphoglycerate kinase, putative	1E-155	At3g12780	1.88
381	382	putative HMG protein	0	At2g17560	1.45
383	384	hypothetical protein	0	At1g76100	1.36
385	386	protein kinase cdc2 homolog B	0	At3g54180	2.39
387	388	T-complex protein 1, beta subunit	0	At5g20890	1.39
389	390	proline oxidase, mitochondrial precursor (osmotic stress-induced)	0	At3g30775	1.45
391	392	linker histone protein, putative	1E-126	At1g14900	1.33
393	394	hypothetical protein	0	At1g27500	2.21
395	396	ARF1-binding protein	0	At5g62010	1.58
397	398	putative protein	0	At5g16270	1.37
399	400	putative protein	1E-173	At5g13850	1.32
401	402	src-2 like protein	0	At1g09070	2.19
403	404	RAN2 small Ras-like GTP-binding nuclear protein (Ran-2)	0	At5g20020	1.3
405	406	phosphoprotein phosphatase (PPX-1)	0	At4g26720	1.42
407	408	nuclear protein-like	0	At5g64270	1.45
409	410	ornithine carbamoyltransferase precursor	0	At1g75330	1.41
411	412	unknown protein	0	At2g41650	1.67
413	414	putative protein	0	At5g17640	1.66
415	416	hypothetical protein	0	At1g57990	2.02
417	418	hypothetical protein	0	At4g15760	1.64
419	420	glycine-rich protein 2 (GRP2)	0	At4g38680	1.72
421	422	hypothetical protein	1E-113	At2g41780	2.6
423	424	RNA-binding protein, putative	8E-95	At3g20250	1.46
425	426	gda-1, putative	2E+00	At3g27090	1.46
427	428	beta-fructofuranosidase 1	0	At3g13790	1.32
429	430	26S proteasome subunit 4-like protein	0	At4g29040	1.51
431	432	putative protein	1E-59	At1g33980	1.42
433	434	hypothetical protein	0	At1g57680	2.66
435	436	unknown protein	0	At1g29980	1.98

437	438	60S ribosomal protein - like	0	At5g02870	1.39
439	440	REVOLUTA or interfascicular fiberless 1	0	At5g60690	1.34
441	442	RAC-like GTP-binding protein ARAC4	1E-180	At1g20090	1.78
443	444	unknown protein	2E-42	At3g07390	1.34
445	446	unknown protein	0	At5g65660	1.7
447	448	unknown protein	1E-154	At3g05040	1.52
449	450	putative DNA gyrase subunit A	1E-153	At3g10690	2.2
451	452	putative protein	0	At3g49170	1.53
453	454	eukaryotic cap-binding protein (gb|AAC17220.1)	0	At5g18110	1.41
455	456	phosphoethanolamine N-methyltransferase, putative	0	At1g73600	1.62
457	458	unknown protein	0	At2g30590	2.78
459	460	RAN1 small Ras-like GTP-binding nuclear protein (Ran-1)	0	At5g20010	1.46
461	462	putative protein	1E-104	At4g24290	1.32
463	464	putative auxin-regulated protein	0	At2g45210	1.33
465	466	adenylosuccinate synthetase	0	At3g57610	1.39
467	468	putative protein	0	At5g14530	2.7
469	470	putative ubiquitin activating enzyme E1 (ECR1)	0	At5g19180	1.63
471	472	putative mitochondrial processing peptidase	0	At3g02090	1.4
473	474	putative protein	0	At3g48530	1.55
475	476	hypothetical protein	0	At1g20330	1.47
477	478	hypothetical protein	0	At4g02590	1.36
479	480	putative pyrophosphate-fructose-6-phosphate 1-phosphotransferase	0	At1g12000	1.49
481	482	putative receptor-like protein kinase	0	At2g02220	1.55
483	484	putative protein	1E-104	At4g02440	1.4
485	486	non-phototropic hypocotyl, putative	0	At1g30440	1.57
487	488	histone deacetylase	0	At5g63110	1.36
489	490	putative protein	0	At5g66580	3.18
491	492	multicatalytic endopeptidase complex, proteasome precursor, beta	0	At4g31300	1.42
493	494	fibrillarin - like protein	6E-77	At4g25630	1.3
495	496	hypothetical protein	8E-45	At1g54060	1.36
497	498	histone H1, partial	0	At2g30620	1.58
499	500	hypothetical protein	0	At3g09030	1.45
501	502	enoyl-CoA hydratase - like protein	0	At4g31810	1.31
503	504	unknown protein	7E+00	At2g27080	1.51
505	506	myb-related transcription factor, putative	0	At3g23250	1.49
507	508	Alcohol Dehydrogenase	0	At1g77120	5.09
509	510	hypothetical protein	1E-132	At1g27590	1.38
511	512	unknown protein	0	At1g14710	1.36
513	514	putative receptor-like protein kinase	0	At2g13790	1.68
515	516	putative protein	0	At5g14550	1.39
517	518	homeobox protein knotted-1 like 4 (KNAT4)	1E-165	At5g11060	1.4
519	520	putative protein	1E-142	At5g15540	1.47
521	522	carbonyl reductase-like protein	7E+00	At5g51030	2.17
523	524	hypothetical protein	1E-50	At1g53900	1.36
525	526	aspartate--tRNA ligase - like protein	0	At4g31180	1.62
527	528	unknown protein	1E-121	At3g06150	1.74
529	530	amino acid transporter protein-like	0	At5g65990	1.59
531	532	12-oxophytodienoate reductase (OPR1)	0	At1g76680	1.43
533	534	calnexin homolog	6E-25	At5g07340	1.39
535	536	unknown protein	0	At1g61100	2.06

537	538	homogentisate 1,2-dioxygenase	1E-78	At5g54080	2.01
539	540	glucosyltransferase -like protein	0	At4g34131	1.33
541	542	putative protein	4E-01	At5g54890	1.35
543	544	hypothetical protein	0	At1g76070	1.31
545	546	putative protein	1E-179	At5g18310	1.56
547	548	DNA binding protein ACBF - like	0	At5g19350	1.36
549	550	hypothetical protein	0	At1g17210	1.69
551	552	putative protein	1E-111	At5g51220	1.46
553	554	RING finger protein	0	At3g61460	2.14
555	556	putative protein	0	At5g18580	1.32
557	558	putative protein kinase	0	At2g31010	1.35
559	560	chloroplast nucleoid DNA binding protein, putative	0	At1g01300	1.49
561	562	unknown protein	1E-143	At1g31130	1.4
563	564	splicing factor, putative	1E+00	At1g14650	1.56
565	566	putative TCP3 gb|AAC24010.	0	At1g53230	1.38
567	568	unknown protein	0	At1g72790	1.71
569	570	ribosomal protein S6 - like	0	At4g31700	1.38
571	572	auxin-resistance protein AXR1	0	At1g05180	1.36
573	574	putative protein	0	At5g11030	1.43
575	576	putative 60S acidic ribosomal protein P0	0	At3g09200	1.47
577	578	mismatch binding protein, putative	0	At3g24320	2.1
579	580	T-complex chaperonin protein , epsilon subunit	0	At1g24510	1.47
581	582	putative protein	0	At4g24120	1.56
583	584	putative protein	4E-38	At5g53900	1.79
585	586	histidine transport protein (PTR2-B)	0	At2g02040	1.37
587	588	unknown protein	0	At3g10490	1.43
589	590	tubulin alpha-5 chain-like protein	0	At5g19770	1.6
591	592	putative non-LTR retroelement reverse transcriptase	6E+00	At2g15510	4.71
593	594	unknown protein	1E-179	At2g41010	1.33
595	596	putative chloroplast outer envelope 86-like protein	0	At4g02510	1.45
597	598	serine/threonine-specific protein kinase NAK	0	At5g02290	1.56
599	600	unknown protein	0	At2g34680	1.45
601	602	hypothetical protein	0	At1g43170	1.69
603	604	phospholipase D, putative, 5' partial	0	At3g16785	1.5
605	606	CTP synthase-like protein	0	At1g30820	1.58
607	608	nitrilase 2	0	At3g44300	1.84
609	610	putative mitogen activated protein kinase kinase	0	At3g04910	1.34
611	612	putative protein	0	At4g27450	1.4
613	614	Phospholipase like protein	0	At4g38550	1.9
615	616	endomembrane-associated protein	3E-41	At4g20260	1.83
617	618	leucine-rich receptor-like protein kinase, putative	0	At1g72180	2.13
619	620	putative protein	8E-01	At4g25930	1.54
621	622	WD-40 repeat protein MSI1 (sp|O22467)	0	At5g58230	1.72
623	624	oxysterol-binding protein - like	1E-171	At5g59420	1.31
625	626	putative protein	1E-178	At4g21840	1.4
627	628	blue copper binding protein	1E-50	At5g20230	2.3
629	630	UV-damaged DNA-binding protein- like	6 E-9	At4g21100	1.46
631	632	fatty acid hydroxylase (FAH1)	0	At2g34770	1.96
633	634	putative thymidine kinase	0	At3g07800	8.44
635	636	hypothetical protein	0	At1g79380	1.41
637	638	unknown protein	0	At2g15860	1.36
639	640	flower pigmentation protein ATAN11	0	At1g12910	1.41

641	642	hypothetical protein	0	At1g56290	1.33
643	644	putative protein	0	At3g62630	1.38
645	646	SNF-2 like RING finger	0	At1g61140	1.42
647	648	unknown protein	0	At3g16310	1.49
649	650	putative glucosyl transferase	0	At2g36800	1.36
651	652	putative protein	0	At4g25170	1.92
653	654	hypothetical protein	9E-39	At4g00450	1.36
655	656	glutathione S-transferase	0	At2g30860	1.49
657	658	unknown protein, 3' partial	0	At3g15095	1.42
659	660	unknown protein	0	At3g21080	1.31
661	662	TCH4 protein (gb|AAA92363.1)	0	At5g57560	1.92
663	664	putative protein	0	At3g61600	1.34
665	666	receptor-like kinase, putative	0	At3g23750	2.06
667	668	putative 2,3-bisphosphoglycerate-independent phosphoglycerate	0	At1g09780	1.34
669	670	putative protein	0	At5g14250	1.51
671	672	DnaJ homologue (gb|AAB91418.1|)	0	At5g06910	2.32
673	674	hypothetical protein	0	At1g33250	1.35
675	676	unknown protein	0	At2g19800	1.81
677	678	aspartate carbamoyltransferase precursor (aspartate	3E-84	At3g20330	1.49
679	680	hypothetical protein	0	At1g16520	1.35
681	682	unknown protein	5E+00	At1g48620	1.33
683	684	putative protein	1E+00	At4g35750	1.39
685	686	hypothetical protein	1E-55	At3g13620	1.79
687	688	RNA helicase, DRH1	1E-179	At3g01540	1.56
689	690	putative 3-oxoacyl [acyl-carrier protein] reductase	0	At1g24360	1.42
691	692	putative cellular apoptosis susceptibility protein	1E-142	At2g46520	1.43
693	694	hypothetical protein	0	At1g01540	1.31
695	696	starch branching enzyme II	2E-61	At2g36390	1.36
697	698	40S ribosomal protein - like	0	At5g15200	1.32
699	700	putative protein	0	At4g13640	1.33
701	702	putative protein	0	At3g45970	3.22
703	704	hypothetical protein	0	At1g66160	1.31
705	706	AP2 domain containing protein RAP2.3	2 E-9	At3g16770	1.51
707	708	putative protein	1E-47	At5g02880	1.32
709	710	NADH-dependent glutamate synthase	0	At5g53460	2.25
711	712	arginine/serine rich splicing factor RSP3	4E-59	At3g61860	1.31
713	714	hypothetical protein	1E-134	At1g55880	1.37
715	716	translation initiation factor eIF3 - like protein	6E-77	At4g20980	1.45
717	718	putative serine/threonine protein phosphatase catalytic subunit,	0	At2g42500	1.38
719	720	unknown protein	1E-105	At1g33480	1.91
721	722	COP1-interacting protein CIP8	0	At5g64920	1.4
723	724	nonphototropic hypocotyl 1	6E+00	At3g45780	1.47
725	726	putative protein	1E-78	At5g10860	1.32
727	728	putative protein	0	At5g19750	1.37
729	730	putative protein	1E-127	At3g52500	1.39
731	732	putative protein	0	At4g10280	1.76
733	734	cytochrome P450 monooxygenase	0	At4g31500	1.35
735	736	ethylene responsive element binding factor	1E-104	At4g17500	1.33
737	738	hypothetical protein	0	At1g17620	1.37
739	740	unknown protein	1E-123	At3g07390	1.42
741	742	putative protein kinase	0	At3g02880	1.46
743	744	DNA repair protein RAD23 homolog	0	At5g38470	1.42

745	746	GTP-binding protein - like	1E-25	At5g03520	1.57
747	748	putative protein	0	At3g63500	1.4
749	750	putative adenylate kinase	4E+00	At2g39270	1.37
751	752	protein kinase - like	6E-46	At5g59010	1.42
753	754	unknown protein	0	At3g04630	1.58
755	756	RNA binding protein	0	At1g73490	1.32
757	758	putative phospholipase D	0	At3g15730	1.51
759	760	importin alpha	1E-115	At3g06720	1.45
761	762	RING-H2 finger protein RHF2a	0	At5g22000	1.43
763	764	putative protein	2E-93	At4g19160	1.3
765	766	putative protein	0	At4g32440	1.41
767	768	putative protein phosphatase type 2C	0	At3g15260	1.61
769	770	putative protein	0	At5g39890	1.31
771	772	ribosomal protein	0	At4g16720	1.42
773	774	dormancy-associated protein	9E+00	At1g28330	2.01
775	776	auxin-inducible gene (IAA2)	0	At3g23030	1.65
777	778	unknown protein	5E+00	At1g76010	1.54
779	780	protein kinase ADK1-like protein	1E+00	At4g28540	1.96
781	782	putative protein	0	At4g24210	1.36
783	784	hypothetical protein	0	At1g79530	1.4
785	786	putative trehalose-6-phosphate synthase	0	At1g68020	1.45
787	788	adenylate kinase	0	At5g63400	1.39
789	790	putative proline-rich protein precursor	0	At1g73840	1.56
791	792	putative protein	5E-87	At5g14370	1.37
793	794	hypothetical protein	0	At4g33290	1.7
795	796	cytochrome P450 monooxygenase (CYP71B3)	0	At3g26220	1.32
797	798	TMV resistance protein N - like	0	At4g19530	1.5
799	800	hypothetical protein	9E-70	At1g54830	1.33
801	802	3-ketoacyl-CoA thiolase	0	At2g33150	1.87
803	804	putative protein	0	At3g54350	1.35
805	806	hypothetical protein	1E-170	At4g02680	1.36
807	808	putative bHLH transcription factor	0	At2g46510	1.35
809	810	RNA-binding protein, putative	5E-84	At3g26420	1.55
811	812	putative lectin	3E-20	At3g09190	1.67
813	814	xyloglucan endotransglycosylase, putative	0	At3g23730	2.85
815	816	unknown protein	2E-33	At2g41170	1.32
817	818	putative protein	3E-78	At3g57150	1.67
819	820	putative glucose regulated repressor protein	0	At2g25490	1.81
821	822	putative AP2 domain containing protein RAP2.4 gi|2281633	1E-150	At1g78080	1.82
823	824	putative sulfate transporter	0	At1g80310	1.51
825	826	G protein alpha subunit 1 (GPA1)	0	At2g26300	1.44
827	828	protochlorophyllide reductase precursor	0	At4g27440	2.39
829	830	Shaggy related protein kinase tetha	0	At4g00720	1.52
831	832	putative protein kinase	0	At3g01300	1.49
833	834	RNA-binding protein-like protein	0	At3g47160	1.31
835	836	unknown protein	1E-150	At5g24670	1.47
837	838	zinc finger protein ZFP8	1E-144	At2g41940	1.42
839	840	GTP binding protein beta subunit	0	At4g34460	1.54
841	842	copia-like retroelement pol polyprotein	0	At2g22680	1.4
843	844	CONSTANS-like B-box zinc finger protein-like	0	At5g57660	1.36
845	846	unknown protein	3E-71	At3g10640	1.33
847	848	putative protein	0	At4g24690	1.91

849	850	NADH dehydrogenase	1E-124	At5g08530	1.42
851	852	unknown protein	0	At1g73820	1.35
853	854	monosaccharide transport protein, STP4	8 E-9	At3g19930	1.58
855	856	globulin-like protein	0	At1g07750	1.61
857	858	putative transitional endoplasmic reticulum ATPase	2E-58	At3g09840	1.51
859	860	putative monodehydroascorbate reductase	0	At1g63940	1.39
861	862	anthranilate phosphoribosyltransferase-like protein	0	At3g57880	1.42
863	864	H+-transporting ATP synthase chain 9 - like protein	6E-25	At4g32260	1.83
865	866	hypothetical protein	0	At1g02810	2.31
867	868	calmodulin-like protein	3E-63	At2g41410	1.52
869	870	putative protein	0	At5g15350	2.75
871	872	glutathione S-transferase	0	At2g30870	1.54
873	874	putative SWI/SNF complex subunit SW13	1E-138	At2g33610	1.32
875	876	MAP kinase kinase 2	0	At4g29810	1.39
877	878	adenosylhomocysteinase	1E-134	At4g13940	2.07
879	880	putative protein	0	At5g27760	1.4
881	882	unknown protein	0	At2g47450	1.67
883	884	putative protein	0	At4g33050	2.2
885	886	50S ribosomal protein L12-C	1E-138	At3g27850	1.38
887	888	26S proteasome AAA-ATPase subunit RPT4a (gb|AAF22524.1)	0	At5g43010	1.4
889	890	unknown protein	8E-01	At3g01690	1.31
891	892	lipid transfer protein; glossy1 homolog	0	At5g57800	1.39
893	894	indoleacetic acid (IAA)-inducible gene (IAA7)	1 E-7	At3g23050	1.52
895	896	histone H2B - like protein	0	At5g59910	2.16
897	898	putative RNA helicase	0	At3g06480	1.47
899	900	unknown protein	8E-64	At1g19310	1.44
901	902	unknown protein	4E-96	At2g18440	1.38
903	904	unknown protein	0	At1g68220	1.59
905	906	unknown protein	1E-142	At2g20570	1.35
907	908	putative replication factor	1E-124	At1g21690	3.3
909	910	U2 snRNP auxiliary factor, small subunit	0	At5g42820	1.55
911	912	replication factor C - like	0	At5g27740	1.45
913	914	nuclear receptor binding factor-like protein	0	At3g45770	1.62
915	916	putative glycosyl transferase	0	At1g24170	2.39
917	918	histone H2A-like protein	4E-53	At5g27670	1.62
919	920	putative protein	1E-125	At5g48960	1.43
921	922	hypothetical protein	0	At1g53740	1.42
923	924	splicing factor - like protein	0	At3g53500	1.39
925	926	unknown protein	0	At1g50510	1.32
927	928	Fe(II) transport protein	0	At4g19690	1.37
929	930	hypothetical protein	0	At1g61730	1.43
931	932	unknown protein	7 E-9	At2g47440	2.5
933	934	cold-regulated protein COR6.6 (KIN2)	0	At5g15970	3.03
935	936	putative cytochrome C	0	At1g22840	1.3
937	938	unknown protein	0	At1g68580	2.13
939	940	putative Ser/Thr protein kinase	0	At1g16270	1.37
941	942	pseudogene	1E-138	At2g25970	2.15
943	944	unknown protein	0	At3g06380	1.67
945	946	Tic22, putative	3E-84	At3g23710	2.14
947	948	unknown protein	0	At1g09250	1.55
949	950	hypothetical protein	0	At1g72930	1.91
951	952	hypothetical protein	2E+00	At1g68820	1.43

953	954	histone H1	0	At2g18050	1.75
955	956	unknown protein	0	At1g08630	1.45
957	958	unknown protein, 5'partial	0	At3g18035	3.31
959	960	unknown protein	0	At1g04140	1.37
961	962	HAL3A protein	0	At3g18030	1.43
963	964	phi-1-like protein	0	At5g64260	3.38
965	966	hypothetical protein	0	At1g12770	1.35
967	968	pollen specific protein SF21	0	At5g56750	1.45
969	970	cysteine proteinase inhibitor like protein	1E-159	At4g16500	1.33
971	972	20S proteasome subunit C8 (PAG1/PRC8 ARATH)	1E-130	At2g27020	1.36
973	974	nodulin-like protein	1E-99	At1g75500	1.34
975	976	hypothetical protein	0	At1g72900	2.04
977	978	hypothetical protein	0	At2g35230	1.42
979	980	arm repeat containing protein homolog	0	At3g46510	1.4
981	982	putative protein	0	At5g67480	1.76
983	984	putative leucyl-tRNA synthetase	1E-118	At1g09620	1.52
985	986	Putative UDP-glucose glucosyltransferase	1E-164	At1g22400	2.34
987	988	alanine aminotransferase, putative	0	At1g17290	1.66
989	990	26S proteasome AAA-ATPase subunit RPT6a	0	At5g19990	1.36
991	992	Ruv DNA-helicase-like protein	0	At5g22330	1.59
993	994	small nuclear ribonucleoprotein, putative	0	At1g65700	1.33
995	996	unknown protein	0	At2g38310	2.79
997	998	protein phosphatase type 1 PP1BG	3E-91	At4g11240	1.51
999	1000	hypothetical protein	3E-41	At2g43410	2.1
1001	1002	putative protein	0	At5g58600	1.42
1003	1004	nodulin-like protein	0	At1g80530	2.07
1005	1006	putative protein	0	At5g56170	1.65
1007	1008	dihydroxyacetone kinase, putative	1E-167	At3g17770	1.67
1009	1010	ribsomal protein - like	1E-155	At5g09770	1.44
1011	1012	101 kDa heat shock protein; HSP101-like protein	0	At5g57710	1.34
1013	1014	unknown protein	0	At5g51340	1.48
1015	1016	unknown protein	0	At3g05730	1.46
1017	1018	putative protein	2E+00	At5g67570	2.6
1019	1020	mitochondrial chaperonin (HSP60)	0	At2g33210	1.75
1021	1022	putative protein	1E-177	At3g63270	1.34
1023	1024	growth factor like protein	0	At4g12720	1.78
1025	1026	RNA helicase, putative	0	At3g19760	1.54
1027	1028	pseudogene	1E-142	At2g34760	1.81
1029	1030	hypothetical protein	0	At3g21740	1.52
1031	1032	shaggy-like kinase beta	0	At3g61160	1.36
1033	1034	unknown protein	1E-165	At1g20100	1.35
1035	1036	24-sterol C-methyltransferase	1E-143	At5g13710	1.41
1037	1038	WD-40 repeat protein (MSI3)	0	At4g35050	4.89
1039	1040	hypothetical protein	0	At1g67120	1.51
1041	1042	putative protein (fragment)	0	At5g14930	1.46
1043	1044	putative protein	1 E-6	At5g54180	1.78
1045	1046	hypothetical protein	1E-126	At1g20570	1.43
1047	1048	calcium-dependent protein kinase	0	At5g66210	2.96
1049	1050	nitrilase 2	1E-127	At3g44300	1.88
1051	1052	methionyl-tRNA synthetase - like protein	1E-173	At4g13780	1.33
1053	1054	putative protein	0	At4g24230	1.58
1055	1056	putative protein	2E-76	At5g19330	1.33

1057	1058	caffeoyl-CoA O-methyltransferase - like protein	1E-166	At4g34050	1.41
1059	1060	putative DNA binding protein	0	At4g27000	1.43
1061	1062	unknown protein	0	At1g55270	1.4
1063	1064	carbamoyl phosphate synthetase large chain (carB)	0	At1g29900	1.5
1065	1066	hypothetical protein	6E+00	At4g02680	2.73
1067	1068	putative RNA helicase	0	At3g22310	1.53
1069	1070	molybdopterin synthase sulphurylase (gb|AAD18050.1)	0	At5g55130	1.77
1071	1072	inner mitochondrial membrane protein, putative	0	At1g17530	1.55
1073	1074	putative protein kinase	0	At3g08760	1.9
1075	1076	putative JUN kinase activator protein	0	At1g22920	1.42
1077	1078	thaumatin, putative	0	At1g75800	1.56
1079	1080	DNA-binding protein	0	At3g14230	1.54
1081	1082	unknown protein	0	At2g01710	1.34
1083	1084	putative calcium binding protein	0	At2g43290	1.57
1085	1086	class 1 non-symbiotic hemoglobin (AHB1)	5E-93	At2g16060	1.86
1087	1088	glycine-rich RNA binding protein, putative	2E-52	At3g23830	1.38
1089	1090	unknown protein	2E-37	At2g01190	1.3
1091	1092	hydoxyethylthiazole kinase, putative	2E-71	At3g24030	1.35
1093	1094	putative protein translocase	0E+00	At2g37410	1.51
1095	1096	putative protein	5E-02	At5g61560	1.31
1097	1098	hypothetical protein	7E-02	At1g35600	1.56
1099	1100	ethylene-insensitive 3	0	At3g20770	1.5
1101	1102	lipoxygenase AtLOX2	0	At3g45140	1.57
1103	1104	putative phosphatidic acid phosphatase	0	At2g01180	1.85
1105	1106	unknown protein	5 E-5	At1g80860	1.3
1107	1108	unknown protein	2 E-15	At3g28180	1.64
1109	1110	LOB domain protien 41	0	At3g02550	4.01
1111	1112	putative protein	2E-02	At5g22260	1.95
1113	1114	actin - like protein	1E-180	At3g60830	1.36
1115	1116	DEAD-box protein abstrakt	0	At5g51280	1.53
1117	1118	putative DNA polymerase epsilon catalytic subunit	2E+00	At2g27120	2.87
1119	1120	unknown protein	6E-59	At5g48020	1.4
1121	1122	protein kinase C inhibitor-like protein	0	At3g56490	1.58
1123	1124	putative PRP19-like spliceosomal protein	0	At2g33340	1.7
1125	1126	germin-like protein	0	At1g72610	1.67
1127	1128	putative protein	1 E-5	At5g10050	1.32
1129	1130	putative protein	0	At4g34950	1.96
1131	1132	zinc finger protein	0	At5g66730	1.37
1133	1134	chaperonin gamma chain - like protein	1E-176	At5g26360	1.67
1135	1136	WD-40 protein	7E+00	At4g07410	1.42
1137	1138	putative DNA-binding protein	0	At4g12080	1.4
1139	1140	beta-glucosidase, putative	0	At1g52400	1.66
1141	1142	hypothetical protein	1E-44	At2g23140	1.66
1143	1144	homeobox protein	1E-43	At3g61150	1.63
1145	1146	glycine-rich protein	0	At4g36020	1.82
1147	1148	unknown protein	0	At3g01460	1.37
1149	1150	hypothetical protein	1E-134	At4g28190	1.4
1151	1152	predicted protein	5E-37	At4g32010	1.34
1153	1154	N-myristoyl transferase	1E-157	At5g57020	1.37
1155	1156	putative protein	0	At4g36780	1.61
1157	1158	unknown protein	2E-01	At5g48240	1.64
1159	1160	unknown protein	0	At1g21630	1.55

1161	1162	unknown protein	1E-102	At1g07360	1.74
1163	1164	lysyl-tRNA synthetase	1E-96	At3g11710	1.38
1165	1166	unknown protein	0	At3g07780	1.51
1167	1168	tryptophan synthase beta chain 1 precursor (sp|P14671)	1E-102	At5g54810	1.55
1169	1170	putative protein	8E-98	At4g25620	1.81
1171	1172	RuvB DNA helicase-like protein	0	At5g67630	1.32
1173	1174	putative pectin methylesterase	0	At3g14310	1.43
1175	1176	putative cytidine deaminase	0	At2g19570	1.41
1177	1178	hypothetical protein	0	At3g12400	1.42
1179	1180	1-aminocyclopropane-1-carboxylate synthase -like protein	0	At4g26200	1.54
1181	1182	peroxidase	3E-88	At2g38380	2.11
1183	1184	2-oxoglutarate dehydrogenase, E1 component	0	At5g65750	1.44
1185	1186	xylosidase	0	At5g49360	1.93
1187	1188	ethylene responsive element binding factor 4	0	At3g15210	1.7
1189	1190	putative protein	2E+00	At5g46650	3.54
1191	1192	eukaryotic protein synthesis initiation factor 4A	0	At3g13920	1.35
1193	1194	Unknown protein	0	At1g76970	2.34
1195	1196	hypothetical protein	0	At1g19380	1.54
1197	1198	unknown protein	0	At5g49640	1.78
1199	1200	putative xyloglucan-specific glucanase	0	At2g01850	1.58
1201	1202	similar to nucellin gb|AAB96882.1	1E-106	At1g49050	1.5
1203	1204	unknown protein	0	At3g29390	1.33
1205	1206	putative protein	0	At3g62190	1.58
1207	1208	putative malate dehydrogenase	0	At1g04410	1.34
1209	1210	putative isocitrate lyase	1E-153	At3g21720	3.08
1211	1212	DNA-binding protein	1E-160	At3g14230	1.48
1213	1214	histone H4-like protein	0	At3g46320	2.55
1215	1216	putative dehydrogenase	0	At1g71170	1.47
1217	1218	alanine--tRNA ligase, putative	0	At1g50200	1.38
1219	1220	oligopeptidase A - like protein	1E-172	At5g10540	1.43
1221	1222	putative protein	0	At5g62620	1.32
1223	1224	permease	0	At5g49990	1.3
1225	1226	DEAD BOX RNA helicase RH15	1E-129	At5g11200	1.4
1227	1228	lipoamide dehydrogenase precursor	1E-128	At3g17240	1.38
1229	1230	hypothetical protein	0	At1g15170	1.75
1231	1232	xyloglucan endo-1,4-beta-D-glucanase (XTR-6)	0	At4g25810	1.95
1233	1234	histone H2B like protein (emb|CAA69025.1)	7E-34	At5g22880	1.91
1235	1236	S-receptor kinase homolog 2 precursor	1E+00	At5g60900	2.61
1237	1238	60S ribosomal protein L2	7E-48	At2g18020	1.58
1239	1240	unknown protein	0	At1g23030	1.98
1241	1242	zinc finger protein, putative	0	At1g34370	1.51
1243	1244	putative protein	3 E-8	At4g05150	1.38
1245	1246	aldose 1-epimerase - like protein	5E-25	At3g47800	1.88
1247	1248	cinnamoyl-CoA reductase - like protein	0	At5g58490	1.35
1249	1250	putative NADP-dependent glyceraldehyde-3-phosphate dehydrogenase	0	At2g24270	1.43
1251	1252	isp4 like protein	0	At4g16370	1.77
1253	1254	putative protein	0	At4g08350	1.32
1255	1256	calmodulin-related protein 2, touch-induced (TCH2)	0	At5g37770	1.55
1257	1258	20S proteasome subunit PAD2 (gb|AAC32059.1)	0	At5g66140	1.5
1259	1260	glucosidase II alpha subunit	0	At5g63840	1.35
1261	1262	putative GAR1 protein	0	At3g03920	1.74

1263	1264	putative protein	3E-45	At5g08450	1.79
1265	1266	glutamate dehydrogenase (EC 1.4.1.-) 1 (pir||S71217)	0	At5g18170	1.47
1267	1268	putative protein	0	At5g06660	1.32
1269	1270	Nonclathrin coat protein gamma - like protein	1E-143	At4g34450	1.43
1271	1272	unknown protein	0	At3g17860	1.6
1273	1274	similar to senescence-associated protein	0	At2g23810	1.59
1275	1276	putative protein	0	At5g60420	1.31
1277	1278	unknown protein	0	At1g28260	1.36
1279	1280	shaggy-like protein kinase etha (EC 2.7.1.-)	0	At4g18710	1.37
1281	1282	putative 26S protease regulatory subunit 6A	0	At1g09100	1.47
1283	1284	unknown protein	0	At3g21140	1.49
1285	1286	dynamin-like protein	0	At2g14120	1.4
1287	1288	scarecrow-like 1	2E-47	At1g21450	1.75
1289	1290	unknown protein	7E-40	At3g02710	1.3
1291	1292	putative protein	0	At5g50670	1.41
1293	1294	helicase-like protein	1E-108	At5g44800	1.5
1295	1296	dynamin-like protein 4 (ADL4)	1E-100	At3g60190	1.32
1297	1298	unknown protein	0	At3g12790	1.31
1299	1300	putative Tub family protein	0	At2g47900	1.37
1301	1302	putative protein	1E-119	At5g13020	1.33
1303	1304	alanine aminotransferase, putative	1E-147	At1g17290	1.36
1305	1306	SCARECROW-like protein	0	At4g36710	1.49
1307	1308	alpha galactosyltransferase-like protein	0	At3g62720	3.26
1309	1310	putative protein	0	At4g31980	1.32
1311	1312	putative protein	1E-124	At3g56480	1.34
1313	1314	histone acetyltransferase HAT B	0	At5g56740	2.36
1315	1316	putative phosphoribosyl pyrophosphate synthetase	3E-97	At2g44530	1.45
1317	1318	AIG1	1E-130	At1g33960	1.45
1319	1320	hypothetical protein	0	At4g22190	1.69
1321	1322	hypothetical protein	0	At1g26180	1.33
1323	1324	putative protein	4E-84	At5g59000	1.61
1325	1326	hypothetical protein	0	At2g27660	1.66
1327	1328	unknown protein	0	At1g33400	1.38
1329	1330	helicase-like protein	0	At5g44800	1.63
1331	1332	putative protein	0	At5g44920	1.43
1333	1334	putative RNA-binding protein	0	At1g22910	2.13
1335	1336	meiosis specific - like protein	0	At5g02820	2.62
1337	1338	isocitrate dehydrogenase - like protein	0	At5g14590	1.43
1339	1340	hypothetical protein	1E-139	At1g15500	1.63
1341	1342	putative protein	3E-01	At5g52270	1.38
1343	1344	ABC transporter-like protein	0	At5g06530	1.63
1345	1346	heat-shock protein 90, putative	0	At1g27640	1.48
1347	1348	unknown protein	0	At3g07220	1.33
2713	2714	large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase		NP_051067	4.71
2715	2716	ribosomal protein L33		NP_051080	3.54
2717	2718	PSII I protein		NP_051074	2.81
2719	2720	ribosomal protein L2		NP_051099	2.61
2721	2722	ATP-dependent protease subunit		NP_051083	2.60
2723	2724	cytochrome B6		NP_051088	2.55
2725	2726	ATPase epsilon subunit		NP_051065	2.17
2728	2729	26S ribosomal RNA protein		NP_085475	2.87

2729	2730	GATA Zn-finger protein		At3g16870	2.75
2731	2732	unknown protein		At5g53740	2.01
2733	2734	putative glucosyltransferase		At2g15480	2.15
2735	2736	Anthocyaninless2		At4g00730	2.73
2737	2738	pectate lyase-like protein		At3g54920	2.13
2739	2740	putative sterol dehydrogenase		At2g43420	2.10

*** This accession number was replaced by a new annotation and called At1g36160

Table 5: Arabidopsis genes 1.3 times (1/ratio) or more repressed in E2Fa/Dpa plants

SEQ ID NO		Gene name	E-value	MIPS accession Number	Ratio
cDNA	PROT				
1349	1350	putative glutathione peroxidase	0	At2g31570	0.51
1351	1352	phenylalanine ammonia lyase (PAL1)	0	At2g37040	0.65
1353	1354	unknown protein	0	At1g04040	0.62
1355	1356	putative protein	0	At4g25340	0.52
1357	1358	water channel - like protein	1E-129	At4g23400	0.7
1359	1360	catalase	0	At4g35090	0.46
1361	1362	stearoyl-ACP desaturase	2E-11	At2g43710	0.54
1363	1364	putative oligopeptide transporter	0	At4g10770	0.37
1365	1366	putative chloroplast 50S ribosomal protein L28	0	At2g33450	0.73
1367	1368	ferredoxin--NADP reductase precursor, putative	0	At1g20020	0.64
1369	1370	3-beta-hydroxysteroid dehydrogenase	1E-44	At2g26260	0.73
1371	1372	putative alanine aminotransferase	1E-127	At1g70580	0.51
1373	1374	hypothetical protein	4E-99	At1g56500	0.66
1375	1376	putative protein	0	At5g21940	0.64
1377	1378	putative protein	1E-158	At5g26970	0.7
1379	1380	actin depolymerizing factor 4 - like protein	0	At5g59890	0.66
1381	1382	hypothetical protein	7E-72	At3g45160	0.5
1383	1384	transporter-like protein	1E-07	At3g53960	0.68
1385	1386	nicotianamine synthase (dbj|BAA74589.1)	0	At5g04950	0.35
1387	1388	cytochrome P450 monooxygenase (CYP83A1)	0	At4g13770	0.39
1389	1390	unknown protein	0	At2g29660	0.77
1391	1392	hypothetical protein	0	At3g12580	0.56
1393	1394	unknown protein	0	At5g64130	0.52
1395	1396	putative protein	0	At3g61870	0.73
1397	1398	fructose-bisphosphate aldolase - like protein	0	At4g26530	0.17
1399	1400	lectin like protein	1E-124	At4g19840	0.74
1401	1402	unknown protein	0	At1g28140	0.72
1403	1404	feebly-like protein	0	At3g01420	0.73
1405	1406	beta-fructosidase	1E-105	At1g62660	0.38
1407	1408	unknown protein	1E-06	At1g15350	0.77
1409	1410	peptidylprolyl isomerase ROC1	0	At4g38740	0.76
1411	1412	hypothetical protein	1E-36	At2g06010	0.74
1413	1414	putative protein	1E-114	At4g30490	0.5
1415	1416	3-isopropylmalate dehydrogenase	0	At5g14200	0.61
1417	1418	putative copper/zinc superoxide dismutase	1E-93	At2g28190	0.77
1419	1420	putative myo-inositol 1-phosphate synthase	0	At2g22240	0.68
1421	1422	putative enolase (2-phospho-D-glycerate hydroylase)	0	At2g29560	0.7
1423	1424	unknown protein	0	At5g43750	0.4
1425	1426	putative protein	1E-22	At4g32330	0.68

1427	1428	putative ferredoxin-thioredoxin reductase	0	At2g04700	0.75
1429	1430	hypothetical protein	1E+00	At3g23290	0.59
1431	1432	putative cellulose synthase	0	At2g32530	0.58
1433	1434	putative protein	0	At5g43850	0.54
1435	1436	putative protein	0	At5g03010	0.58
1437	1438	hypothetical protein	0	At1g78140	0.61
1439	1440	unknown protein	0	At1g72590	0.35
1441	1442	hypothetical protein	0	At1g54450	0.59
1443	1444	hypothetical protein	0	At1g19110	0.73
1445	1446	endo-beta-1,4-glucanase, putative	0	At1g75680	0.7
1447	1448	unknown protein	0	At1g63010	0.76
1449	1450	hypothetical protein	2E-58	At4g24700	0.57
1451	1452	glyoxalase II	0	At1g53580	0.65
1453	1454	putative protein	0	At3g52370	0.53
1455	1456	unknown protein	0	At1g80280	0.57
1457	1458	protein phosphatase ABI1	0	At4g26080	0.71
1459	1460	33 kDa polypeptide of oxygen-evolving complex (OEC) in photosystem	1E-115	At5g66570	0.65
1461	1462	beta-xylosidase	1E-163	At5g64570	0.55
1463	1464	GDP-mannose pyrophosphorylase	0	At2g39770	0.62
1465	1466	peroxidase ATP20a (emb|CAA67338.1)	0	At5g14130	0.67
1467	1468	putative glutathione transferase	0	At1g17190	0.71
1469	1470	putative protein	0	At4g38080	0.75
1471	1472	unknown protein	1E-179	At1g61190	0.7
1473	1474	50S ribosomal protein L24, chloroplast precursor	0	At5g54600	0.76
1475	1476	unknown protein	1E-179	At1g68260	0.55
1477	1478	subtilisin-like serine proteinase, putative, 3' partial	0	At3g14067	0.62
1479	1480	putative protein	0	At4g23890	0.59
1481	1482	unknown protein	0	At3g01690	0.7
1483	1484	putative protein	0	At3g56290	0.3
1485	1486	unknown protein	0	At2g39450	0.67
1487	1488	unknown protein	0	At5g64130	0.66
1489	1490	putative protein	0	At4g30140	0.54
1491	1492	ribulose bisphosphate carboxylase small chain 3b precursor (RuBisCO	1E-145	At5g38410	0.54
1493	1494	Myb DNA binding protein -like	0	At3g46130	0.75
1495	1496	putative 2-cys peroxiredoxin	0	At3g11630	0.64
1497	1498	putative trypsin inhibitor	0	At1g73260	0.59
1499	1500	O-methyltransferase	1E-127	At5g54160	0.62
1501	1502	hypothetical protein	2E-30	At1g29270	0.73
1503	1504	RP19 gene for chloroplast ribosomal protein CL9	9E-67	At3g44890	0.68
1505	1506	putative phosphoglyceride transfer protein	1E-178	At4g08690	0.57
1507	1508	putative protein	0	At5g63530	0.53
1509	1510	putative protein	0	At5g38720	0.68
1511	1512	hypothetical protein	0	At1g72030	0.68
1513	1514	unknown protein	9E-21	At5g09990	0.67
1515	1516	zinc finger protein ZAT7	0	At3g46090	0.73
1517	1518	putative nodulin	0	At3g05180	0.64
1519	1520	putative wound-induced basic protein	1E-160	At3g07230	0.75
1521	1522	hypothetical protein	0	At4g02920	0.38
1523	1524	putative protein	1E-154	At5g62220	0.73
1525	1526	myosin heavy chain-like protein	0	At3g16000	0.5

1527	1528	unknown protein	0	At1g09610	0.76
1529	1530	arabinogalactan protein - like	0	At5g03170	0.71
1531	1532	biotin carboxyl carrier protein of acetyl-CoA carboxylase precursor	0	At5g16390	0.69
1533	1534	centrin	0	At3g50360	0.74
1535	1536	vegetative storage protein Vsp1	0	At5g24780	0.48
1537	1538	protein kinase, putative	1E-61	At1g52310	0.63
1539	1540	unknown protein	1E-132	At2g42760	0.63
1541	1542	phenylalanine ammonia lyase (PAL1)	0	At2g37040	0.72
1543	1544	UDP rhamnose-anthocyanidin-3-glucoside rhamnosyltransferase - like	0	At4g27560	0.45
1545	1546	unknown protein	0	At2g17500	0.54
1547	1548	NAC domain protein, putative	0	At1g01720	0.72
1549	1550	ubiquitin-conjugating enzyme-like protein	2E-24	At5g56150	0.41
1551	1552	putative RNA-binding protein	1E-136	At2g37220	0.72
1553	1554	Overlap with bases 87,142-90,425 of 'IGF' BAC clone F9K20, accession	0	At1g78570	0.52
1555	1556	hypothetical protein	1E-105	At2g04040	0.52
1557	1558	Isp4-like protein	4E-01	At5g64410	0.39
1559	1560	ids4-like protein	0	At5g20150	0.58
1561	1562	unknown protein	3E-98	At1g44000	0.67
1563	1564	R2R3-MYB transcription factor	0	At3g50060	0.66
1565	1566	putative hexose transporter	0	At4g02050	0.68
1567	1568	one helix protein (OHP)	0	At5g02120	0.57
1569	1570	UDP-glucose dehydrogenase-like protein	0	At5g15490	0.74
1571	1572	putative protein	0	At3g54260	0.63
1573	1574	putative L5 ribosomal protein	0	At4g01310	0.75
1575	1576	putative myosin heavy chain	0	At2g37080	0.61
1577	1578	clpB heat shock protein-like	0	At5g15450	0.57
1579	1580	unknown protein	4E-71	At1g52510	0.66
1581	1582	beta-fructosidase, putative	0	At1g12240	0.55
1583	1584	hypothetical protein	0	At1g47670	0.69
1585	1586	putative protein	3E-36	At5g25890	0.75
1587	1588	predicted protein	1E-108	At4g31390	0.73
1589	1590	putative phospholipase	0	At2g39420	0.66
1591	1592	ATP-dependent transmembrane transporter, putative	0	At1g51460	0.74
1593	1594	H+-transporting ATP synthase-like protein	0	At4g09650	0.64
1595	1596	putative protein	0	At4g29590	0.77
1597	1598	unknown protein	0	At3g02640	0.49
1599	1600	phosphoenolpyruvate carboxylase (PPC)	0	At3g14940	0.77
1601	1602	pollen allergen-like protein	0	At1g24020	0.28
1603	1604	putative AUX1-like permease	0	At1g77690	0.73
1605	1606	putative protein	1E-127	At4g39730	0.49
1607	1608	homeobox-leucine zipper protein ATHB-12	0	At3g61890	0.24
1609	1610	putative protein	0	At5g10160	0.53
1611	1612	unknown protein	0	At1g71480	0.56
1613	1614	putative violaxanthin de-epoxidase precursor (U44133)	0	At1g08550	0.7
1615	1616	nClpP5, putative	0	At1g49970	0.68
1617	1618	hypothetical protein	0	At1g65260	0.57
1619	1620	putative protein	1E-135	At3g52360	0.38
1621	1622	putative protein	0	At5g26260	0.5
1623	1624	unknown protein	0	At1g25170	0.66
1625	1626	hypothetical protein	0	At1g79550	0.65

1627	1628	tubulin beta-2/beta-3 chain (sp|P29512)	2E-21	At5g62700	0.61
1629	1630	eukaryotic translation initiation factor 4E, putative	0	At1g29550	0.64
1631	1632	transport inhibitor response 1, putative	1E-175	At1g12820	0.77
1633	1634	osmotin precursor	1E-110	At4g11650	0.74
1635	1636	putative glutathione S-transferase TSI-1	0	At1g10360	0.72
1637	1638	protein ch-42 precursor, chloroplast	0	At4g18480	0.76
1639	1640	omega-3 fatty acid desaturase	2E-06	At2g29980	0.73
1641	1642	unknown protein	0	At2g44670	0.57
1643	1644	putative protein	0	At3g55330	0.51
1645	1646	putative calmodulin	0	At3g51920	0.55
1647	1648	plastid ribosomal protein L34 precursor, putative	1E-140	At1g29070	0.69
1649	1650	putative protein	0	At5g67070	0.66
1651	1652	putative 2Fe-2S iron-sulfur cluster protein	0	At3g16250	0.69
1653	1654	hypothetical protein	0	At1g42970	0.69
1655	1656	hypothetical protein	3E-69	At3g14190	0.6
1657	1658	thylakoid luminal protein	1E-122	At1g77090	0.7
1659	1660	putative protein	0	At3g48420	0.42
1661	1662	actin 3	0	At2g37620	0.64
1663	1664	OEP8 like protein	4E-38	At4g15800	0.73
1665	1666	putative Ras-like GTP-binding protein	0	At3g09910	0.71
1667	1668	sulfolipid biosynthesis protein SQD1	0	At4g33030	0.68
1669	1670	oleosin isoform	0	At3g27660	0.61
1671	1672	acyl-CoA synthetase, putative	0	At1g64400	0.59
1673	1674	putative protein	1E-147	At3g61060	0.5
1675	1676	hypothetical protein	1E-117	At1g56200	0.64
1677	1678	putative protein	0	At4g13500	0.53
1679	1680	cinnamoyl CoA reductase, putative	0	At1g80820	0.72
1681	1682	hypothetical protein	1E-157	At4g28410	0.1
1683	1684	hypothetical protein	0	At1g54030	0.68
1685	1686	putative DNA-binding protein, GT-1	0	At3g25990	0.1
1687	1688	germin-like protein	3E-04	At3g05950	0.49
1689	1690	putative glutathione S-transferase	0E+00	At2g29480	0.7
1691	1692	arabinogalactan-protein (gb|AAC77823.1)	1E-06	At5g64310	0.61
1693	1694	periaxin - like protein	1E-151	At5g09530	0.71
1695	1696	zeaxanthin epoxidase precursor	0	At5g67030	0.52
1697	1698	putative photosystem I reaction center subunit IV	0	At2g20260	0.7
1699	1700	putative 60S ribosomal protein L18A	0	At3g14600	0.74
1701	1702	putative ethylene response element binding protein (EREBP)	0	At2g44840	0.72
1703	1704	unknown protein	0	At2g21970	0.5
1705	1706	RNA-binding protein cp33 precursor	0	At3g52380	0.73
1707	1708	unknown protein	1E-152	At2g34460	0.62
1709	1710	CONSTANS-like 1	1E-179	At5g15850	0.6
1711	1712	unknown protein	0	At1g75100	0.77
1713	1714	ion channel	9E-66	At1g15990	0.57
1715	1716	unknown protein	0	At2g21960	0.46
1717	1718	unknown protein	0	At1g66330	0.69
1719	1720	putative protein	0	At4g26630	0.68
1721	1722	unknown protein	1E-99	At3g28230	0.72
1723	1724	hypothetical protein	1E-65	At1g55910	0.65
1725	1726	putative Na+-dependent inorganic phosphate cotransporter	0	At2g29650	0.52
1727	1728	hypothetical protein	4E-23	At1g02330	0.71
1729	1730	hypothetical protein	0	At1g29700	0.55

1731	1732	putative flavonol 3-O-glucosyltransferase	0	At2g18560	0.62
1733	1734	lycopene epsilon cyclase	0	At5g57030	0.6
1735	1736	hypothetical protein	0	At3g09150	0.75
1737	1738	putative protein	1E-150	At1g31710	0.5
1739	1740	hypothetical protein	0	At1g78850	0.69
1741	1742	putative protein	0	At4g32770	0.75
1743	1744	putative protein	2E-77	At4g22890	0.75
1745	1746	ripening-related protein - like	0	At5g20740	0.59
1747	1748	putative peroxidase ATP12a	0	At1g05240	0.65
1749	1750	hypothetical protein	7E-18	At4g01050	0.77
1751	1752	V-ATPase subunit G (vag2 gene)	4E-04	At4g23710	0.61
1753	1754	hypothetical protein	0	At1g58080	0.75
1755	1756	putative protein	2E-94	At5g19190	0.51
1757	1758	hypothetical protein	0	At1g48850	0.69
1759	1760	putative protein	0	At4g38800	0.75
1761	1762	similar to polygalacturonase-like protein	0	At1g10640	0.28
1763	1764	putative glutathione S-transferase	0	At2g02390	0.73
1765	1766	putative calcium-binding EF-hand protein	3E-78	At2g33380	0.69
1767	1768	unknown protein	1E-113	At1g64680	0.57
1769	1770	unknown protein	0	At3g15660	0.58
1771	1772	putative protein	0	At5g22080	0.74
1773	1774	high mobility group protein 2-like	2E-24	At3g51880	0.71
1775	1776	similar to late embryogenesis abundant proteins	4E-50	At2g44060	0.61
1777	1778	putative protein	0	At4g34600	0.74
1779	1780	putative protein	2E-31	At5g52060	0.48
1781	1782	NADPH oxidoreductase, putative	0	At1g75280	0.53
1783	1784	hypothetical protein	0	At1g16720	0.62
1785	1786	unknown protein	0	At3g28130	0.75
1787	1788	glutaredoxin	0	At4g15690	0.73
1789	1790	putative protein	4E-01	At3g47590	0.66
1791	1792	putative protein	0	At4g26630	0.7
1793	1794	putative polyprotein	1E-139	At4g04410	0.76
1795	1796	MTN3-like protein	0	At3g48740	0.49
1797	1798	hypothetical protein	0	At1g32900	0.38
1799	1800	unknown protein	0	At2g33180	0.77
1801	1802	hypothetical protein	0	At1g66890	0.69
1803	1804	unknown protein	0	At1g74730	0.74
1805	1806	putative ribosomal protein S9	1E-122	At1g74970	0.7
1807	1808	phenylalanine ammonia-lyase	3E-51	At3g53260	0.53
1809	1810	unknown protein	2E-27	At1g78110	0.76
1811	1812	unknown protein	0	At1g18300	0.75
1813	1814	putative prolylcarboxypeptidase	1E-174	At2g24280	0.64
1815	1816	unknown protein	1E-12	At3g24100	0.76
1817	1818	unknown protein	0	At3g18990	0.39
1819	1820	hypothetical protein	1E-127	At1g78890	0.75
1821	1822	unknown protein	5E-87	At2g21530	0.71
1823	1824	hypothetical protein	1E-172	At1g20340	0.71
1825	1826	putative glucosyltransferase	0	At2g31790	0.63
1827	1828	allergen like protein	1E-129	At4g17030	0.74
1829	1830	unknown protein	0	At1g73750	0.72
1831	1832	APG5 (autophagy 5)-like protein	0	At5g17290	0.7
1833	1834	putative protochlorophyllide reductase	0	At1g03630	0.57

1835	1836	zinc finger protein, putative	0	At3g19580	0.61
1837	1838	unknown protein	0	At2g35190	0.65
1839	1840	phosphate/triose-phosphate translocator precursor (gb|AAC83815.1)	4E-33	At5g46110	0.73
1841	1842	unknown protein	0	At5g50840	0.77
1843	1844	hypothetical protein	0	At4g34090	0.69
1845	1846	hypothetical protein	0	At1g14340	0.64
1847	1848	unknown protein	0	At1g67860	0.42
1849	1850	tyrosine transaminase like protein	1E-180	At4g23600	0.47
1851	1852	unknown protein	1E-173	At1g53890	0.53
1853	1854	pectinesterase, putative	0	At1g41830	0.76
1855	1856	putative protein	4E-72	At5g45550	0.69
1857	1858	putative ligand-gated ion channel subunit	2E+00	At2g32400	0.45
1859	1860	unknown protein	0	At3g19370	0.42
1861	1862	putative protein	5E-13	At5g62580	0.59
1863	1864	putative protein	0	At3g61080	0.42
1865	1866	putative squamosa-promoter binding protein 2	1E-162	At1g27360	0.74
1867	1868	sucrose-phosphate synthase - like protein	0	At4g10120	0.22
1869	1870	hypothetical protein	4E-23	At1g62180	0.43
1871	1872	ribosomal protein	0	At4g15000	0.75
1873	1874	MYB-related transcription factor (CCA1)	0	At2g46830	0.46
1875	1876	pinoresinol-lariciresinol reductase, putative	1E-124	At1g32100	0.72
1877	1878	putative protein	0	At3g52230	0.71
1879	1880	3-keto-acyl-CoA thiolase 2 (gb|AAC17877.1)	0	At5g48880	0.57
1881	1882	putative protein	0	At3g46780	0.63
1883	1884	DNA-binding protein, putative	0	At1g01060	0.62
1885	1886	putative protein	3E-20	At4g30990	0.6
1887	1888	putative protein	0	At3g46780	0.59
1889	1890	hypothetical protein	1E-174	At1g28400	0.58
1891	1892	DNA binding protein - like	0	At5g61600	0.55
1893	1894	putative protein	0	At3g62260	0.72
1895	1896	putative CCCH-type zinc finger protein	0	At2g25900	0.63
1897	1898	ubiquitin-conjugating enzyme E2-17 kD 8 (ubiquitin-protein ligase	3E-16	At5g41700	0.42
1899	1900	microbody NAD-dependent malate dehydrogenase	0	At5g09660	0.63
1901	1902	glyceraldehyde 3-phosphate dehydrogenase A subunit (GapA)	0	At3g26650	0.63
1903	1904	microbody NAD-dependent malate dehydrogenase	0	At5g09660	0.66
1905	1906	sedoheptulose-bisphosphatase precursor	0	At3g55800	0.54
1907	1908	putative Fe(II) transporter	1E-175	At2g32270	0.74
1909	1910	germin - like protein	0	At5g38940	0.75
1911	1912	putative malonyl-CoA:Acyl carrier protein transacylase	0	At2g30200	0.7
1913	1914	hypothetical protein	0	At1g19000	0.61
1915	1916	FRO1-like protein; NADPH oxidase-like	0	At5g49740	0.41
1917	1918	J8-like protein	0	At1g80920	0.51
1919	1920	putative protein	0	At4g34190	0.63
1921	1922	photosystem II stability/assembly factor HCF136 (sp|O82660)	0	At5g23120	0.66
1923	1924	hypothetical protein	0	At4g24930	0.63
1925	1926	2-cys peroxiredoxin-like protein	0	At5g06290	0.69
1927	1928	putative protein	0	At3g53470	0.54
1929	1930	unknown protein	3E-96	At3g02180	0.71
1931	1932	F12P19.7	0	At1g65900	0.69
1933	1934	putative fibrillin	0	At4g04020	0.28
1935	1936	putative protein	1E-01	At4g18810	0.72

1937	1938	hypothetical protein	1E-171	At1g50240	0.67
1939	1940	putative protein	0	At3g63210	0.76
1941	1942	unknown protein	0	At2g32870	0.47
1943	1944	Glucose-1-phosphate adenylyltransferase (ApL1/adg2)	0	At5g19220	0.64
1945	1946	unknown protein	1E-66	At2g46100	0.67
1947	1948	farnesyl diphosphate synthase precursor (gb|AAB49290.1)	0	At5g47770	0.71
1949	1950	pyridoxine biosynthesis protein - like	0	At5g01410	0.47
1951	1952	hypothetical protein	0	At4g03820	0.71
1953	1954	putative myrosinase-binding protein	1E-47	At2g39310	0.38
1955	1956	unknown protein	0	At1g05870	0.44
1957	1958	heat shock protein, putative	0	At1g06460	0.28
1959	1960	RIBOSOMAL PROTEIN, putative	1E-175	At1g71720	0.76
1961	1962	elongation factor G, putative	0	At1g62750	0.65
1963	1964	mitochondrial Lon protease homolog 1 precursor (sp|O64948)	0	At5g47040	0.76
1965	1966	cytochrome c	2E-37	At4g10040	0.72
1967	1968	hypothetical protein	1E-102	At4g03420	0.69
1969	1970	putative DnaJ protein	1E-160	At2g41000	0.73
1971	1972	hypothetical protein	0	At2g27290	0.61
1973	1974	putative protein	1E-117	At5g50100	0.4
1975	1976	phytoene synthase (gb|AAB65697.1)	0	At5g17230	0.64
1977	1978	putative protein	0	At4g28230	0.73
1979	1980	hypothetical protein	0	At2g01260	0.49
1981	1982	unknown protein	0	At3g17520	0.71
1983	1984	Ran binding protein (AtRanBP1b)	0	At2g30060	0.73
1985	1986	putative protein	0	At4g32190	0.63
1987	1988	unknown protein	0	At1g19400	0.64
1989	1990	sucrose-phosphate synthase-like protein	0	At5g20280	0.67
1991	1992	putative protein	1E-136	At5g03545	0.45
1993	1994	biotin carboxyl carrier protein precursor-like protein	1E-124	At5g15530	0.54
1995	1996	unknown protein	4E-85	At1g16320	0.53
1997	1998	unknown protein	5E-16	At3g32930	0.68
1999	2000	putative protein	1E-142	At4g35290	0.74
2001	2002	glutathione S-transferase-like protein	0	At5g17220	0.66
2003	2004	fructose 1,6-bisphosphatase, putative	0	At1g43670	0.63
2005	2006	peptidylprolyl isomerase-like protein	2E-34	At5g13120	0.72
2007	2008	teosinte branched1 - like protein	0	At4g18390	0.63
2009	2010	putative protein	0	At3g51520	0.71
2011	2012	lactoylglutathione lyase-like protein	0	At1g11840	0.45
2013	2014	late embryogenesis abundant protein LEA like	0	At5g06760	0.55
2015	2016	putative protein	1E-177	At5g19590	0.71
2017	2018	putative protein	0	At3g63190	0.72
2019	2020	hypothetical protein	0	At1g69510	0.47
2021	2022	putative protein kinase	0	At2g30040	0.69
2023	2024	xyloglucan endo-transglycosylase	0	At3g44990	0.43
2025	2026	phospholipid hydroperoxide glutathione peroxidase	0	At4g11600	0.71
2027	2028	sedoheptulose-bisphosphatase precursor	0	At3g55800	0.51
2029	2030	Clp proteinase like protein	2E-55	At4g17040	0.75
2031	2032	unknown protein	0	At5g07020	0.68
2033	2034	unknown protein	2E-32	At5g51720	0.49
2035	2036	endomembrane protein, putative	1E-117	At1g14670	0.75
2037	2038	putative phosphomannomutase	0	At2g45790	0.66
2039	2040	putative protein	1E-95	At4g27280	0.46

2041	2042	mrp protein, putative	0	At3g24430	0.75
2043	2044	putative vacuolar ATPase	0	At4g02620	0.74
2045	2046	phosphate transporter, putative	0	At3g26570	0.61
2047	2048	similar to Trp Asp repeat protein emb|CAB39845.1; similar to EST	0	At1g78070	0.74
2049	2050	putative MAP kinase	2E-18	At2g01450	0.51
2051	2052	ethylene-responsive transcriptional coactivator, putative	0	At3g24500	0.51
2053	2054	6-phosphogluconolactonase-like protein	0	At5g24420	0.52
2055	2056	beta-amylase-like proten	1E-175	At5g18670	0.4
2057	2058	hypothetical protein	3E-53	At1g20970	0.72
2059	2060	chloroplast 50S ribosomal protein L31, putative	0	At1g75350	0.74
2061	2062	cytochrome P450-like protein	0	At4g37320	0.67
2063	2064	putative potassium transporter AtKT5p (AtKT5)	0	At4g33530	0.76
2065	2066	putative ribosomal-protein S6 kinase (ATPK6)	0	At3g08730	0.63
2067	2068	hypothetical protein	0	At1g04770	0.68
2069	2070	transcription factor Hap5a	6E-74	At3g48590	0.6
2071	2072	putative protein	0	At5g20070	0.69
2073	2074	beta-expansin	0	At2g20750	0.72
2075	2076	SOUL-like protein	4E-82	At1g17100	0.71
2077	2078	unknown protein	0	At1g70760	0.4
2079	2080	unknown protein	1E-124	At2g20890	0.73
2081	2082	unknown protein	1E-160	At1g07280	0.72
2083	2084	unknown protein	0	At1g64680	0.65
2085	2086	ADPG pyrophosphorylase small subunit (gb|AAC39441.1)	0	At5g48300	0.68
2087	2088	unknown protein	0	At2g17340	0.61
2089	2090	hypothetical protein	0	At1g26800	0.74
2091	2092	unknown protein	0	At1g22930	0.67
2093	2094	polyphosphoinositide binding protein, putative	0	At1g01630	0.72
2095	2096	caffeoyl-CoA O-methyltransferase - like protein	0	At4g34050	0.67
2097	2098	pectinesterase	0	At5g53370	0.56
2099	2100	unknown protein	7E-75	At1g64370	0.43
2101	2102	p-nitrophenylphosphatase-like protein	0	At5g36790	0.52
2103	2104	putative protein	1E-172	At5g55960	0.64
2105	2106	serine/threonine protein kinase -like protein	0	At5g10930	0.26
2107	2108	cytosolic factor, putative	0	At1g72160	0.67
2109	2110	S-adenosylmethionine:2-demethylmenaquinone methyltransferase-like	1E-159	At5g56260	0.76
2111	2112	pectate lyase	0	At5g63180	0.67
2113	2114	vacuolar sorting receptor-like protein	0	At4g20110	0.7
2115	2116	putative membrane channel protein	0	At2g28900	0.76
2117	2118	putative thylakoid lumen rotamase	0	At3g01480	0.56
2119	2120	putative chloroplast prephenate dehydratase	0	At3g44720	0.73
2121	2122	3-oxoacyl-[acyl-carrier-protein] synthase I precursor	0	At5g46290	0.76
2123	2124	P-Protein - like protein	1E-108	At4g33010	0.73
2125	2126	NHE1 Na+/H+ exchanger	1E-122	At5g27150	0.73
2127	2128	receptor kinase-like protein	0	At3g47580	0.72
2129	2130	raffinose synthase -like protein	0	At5g40390	0.59
2131	2132	thylakoid luminal protein	0	At1g54780	0.63
2133	2134	unknown protein	0	At2g46170	0.73
2135	2136	beta-xylan endohydrolase -like protein	9E-02	At4g33810	0.26
2137	2138	putative protein	1E-137	At4g12700	0.6
2139	2140	putative ribose 5-phosphate isomerase	0	At3g04790	0.76
2141	2142	putative protein	0	At5g47840	0.7

2143	2144	putative RNA-binding protein	0	At1g09340	0.57
2145	2146	adenine phosphoribosyltransferase (EC 2.4.2.7) - like protein	0	At4g22570	0.46
2147	2148	unknown protein	0	At3g15950	0.37
2149	2150	putative glutathione peroxidase	7E-12	At2g25080	0.46
2151	2152	putative protein	0	At5g23060	0.63
2153	2154	pectate lyase 1-like protein	0	At1g67750	0.42
2155	2156	putative triosephosphate isomerase	9E-61	At2g21170	0.66
2157	2158	carbonate dehydratase - like protein	0	At4g33580	0.72
2159	2160	putative protein	0	At5g37300	0.56
2161	2162	putative protein	1E-143	At3g60080	0.77
2163	2164	cystatin (emb|CAA03929.1)	2E-83	At5g12140	0.74
2165	2166	putative cytochrome b5	0	At2g46650	0.46
2167	2168	putaive DNA-binding protein	2E-08	At4g31550	0.63
2169	2170	hypothetical protein	1E-143	At3g21050	0.5
2171	2172	putative beta-hydroxyacyl-ACP dehydratase	0	At2g22230	0.59
2173	2174	2-oxoglutarate/malate translocator	0	At5g64290	0.77
2175	2176	hypothetical protein	1E-123	At3g27050	0.49
2177	2178	putative alcohol dehydrogenase	9E-64	At2g37770	0.64
2179	2180	hypothetical protein	1E-107	At1g18730	0.67
2181	2182	putative pectinacetylesterase	0	At4g19420	0.71
2183	2184	similar to ADP-ribosylation factor gb|AAD17207; similar to ESTs	2E-80	At1g10630	0.67
2185	2186	hypothetical protein	0	At1g04420	0.67
2187	2188	putative protein	0	At4g26710	0.62
2189	2190	putative protein	0	At4g34630	0.72
2191	2192	latex protein	0	At1g70890	0.29
2193	2194	RCc3- like protein	0	At4g22490	0.57
2195	2196	hypothetical protein	5E-53	At1g20450	0.49
2197	2198	glucosyltransferase-like protein	3E-31	At5g22740	0.65
2199	2200	glutathione S-transferase	0	At2g29450	0.52
2201	2202	putative protein	0	At3g44450	0.59
2203	2204	cysteine synthase	0	At5g28020	0.6
2205	2206	ATP synthase	0	At4g04640	0.57
2207	2208	40S ribosomal protein S14	1E-25	At2g36160	0.67
2209	2210	putative protein	0	At4g19100	0.76
2211	2212	K Efflux antiporter KEA1	0	At1g01790	0.65
2213	2214	hypothetical protein	1E-169	At2g42980	0.66
2215	2216	cytochrome P450 like protein	1E-01	At4g36380	0.48
2217	2218	unknown protein	8E-64	At2g01520	0.23
2219	2220	hypothetical protein	1E-157	At1g07130	0.66
2221	2222	putative protein	5E-04	At5g09620	0.62
2223	2224	unknown protein	0	At1g08470	0.66
2225	2226	putative protein	6E-37	At3g54600	0.7
2227	2228	DnaJ - like protein	1E-68	At4g39960	0.52
2229	2230	putative protein phosphatase 2C	1E-161	At1g78200	0.72
2231	2232	biotin synthase (Bio B)	0	At2g43360	0.67
2233	2234	unknown protein	3E-69	At3g17510	0.55
2235	2236	high mobility group protein 2-like	1E-107	At3g51880	0.66
2237	2238	putative proline-rich protein	0	At2g21140	0.57
2239	2240	cyclin delta-3	0	At4g34160	0.74
2241	2242	serine carboxypeptidase II - like protein	0	At4g30810	0.77
2243	2244	unknown protein	0	At1g67330	0.7
2245	2246	putative protein	7E-93	At3g56010	0.7

2247	2248	GTP-binding protein LepA homolog	0	At5g08650	0.76
2249	2250	unknown protein	0	At3g10420	0.42
2251	2252	putative protein	0	At3g51510	0.58
2253	2254	putative protein	0	At3g45870	0.73
2255	2256	putative enolase	0	At1g74030	0.65
2257	2258	putative protein	3E-05	At5g11680	0.71
2259	2260	putative protein	0	At5g26280	0.58
2261	2262	O-methyltransferase, putative	0	At1g21100	0.63
2263	2264	beta-1,3-glucanase class I precursor	0	At4g16260	0.51
2265	2266	protein phosphatase 2C (PP2C)	2E-27	At3g11410	0.67
2267	2268	root cap protein 2-like protein	1E-174	At5g54370	0.75
2269	2270	putative adenosine phosphosulfate kinase	0	At2g14750	0.47
2271	2272	putative protein	0	At4g30010	0.73
2273	2274	putative uroporphyrinogen decarboxylase	2 E-9	At2g40490	0.75
2275	2276	putative protein	1E-151	At3g57400	0.71
2277	2278	branched-chain amino acid aminotransferase, putative	1E-56	At3g19710	0.3
2279	2280	copia-like retroelement pol polyprotein	0	At2g19830	0.72
2281	2282	neoxanthin cleavage enzyme-like protein	0	At4g19170	0.38
2283	2284	hypothetical protein	0	At1g31860	0.7
2285	2286	unknown protein	0	At2g26570	0.61
2287	2288	asparagine synthetase ASN3	0	At5g10240	0.72
2289	2290	hypothetical protein	1E-80	At1g64770	0.56
2291	2292	expansin S2 precursor, putative	1E-114	At1g20190	0.51
2293	2294	5'-adenylylsulfate reductase	0	At4g04610	0.43
2295	2296	putative protein	9E-02	At3g59680	0.71
2297	2298	putative MYB family transcription factor	4E-31	At2g37630	0.73
2299	2300	Putative protein kinase	3E-23	At1g51850	0.6
2301	2302	putative protein	0	At5g15910	0.76
2303	2304	AALP protein	0	At5g60360	0.63
2305	2306	putative galactinol synthase	0	At2g47180	0.69
2307	2308	cyanohydrin lyase like protein	0	At4g16690	0.56
2309	2310	putative protein	0	At5g03880	0.57
2311	2312	putative glucosyltransferase	0	At2g30150	0.73
2313	2314	cysteine endopeptidase precursor - like protein	0	At3g48350	0.65
2315	2316	unknown protein	1E-122	At3g07700	0.7
2317	2318	putative peroxiredoxin	2E-86	At3g26060	0.76
2319	2320	MAPKK	0	At1g73500	0.58
2321	2322	hypothetical protein	7E-74	At1g64780	0.52
2323	2324	UDP glucose:flavonoid 3-o-glucosyltransferase, putative	2E-90	At1g30530	0.59
2325	2326	hypothetical protein	0	At4g02800	0.55
2327	2328	oxidoreductase -like protein	0	At3g55290	0.65
2329	2330	hypothetical protein	0	At1g50670	0.73
2331	2332	carnitine/acylcarnitine translocase-like protein	0	At5g46800	0.58
2333	2334	MATH protein	1E-169	At4g00780	0.57
2335	2336	unknown protein	0	At1g22630	0.76
2337	2338	cytochrome P450-like protein	0	At4g37330	0.72
2339	2340	putative endo-1,4-beta glucanase	8E-36	At4g02290	0.62
2341	2342	hevein-like protein precursor	0E+00	At3g04720	0.75
2343	2344	leucine zipper-containing protein AT103	1E-139	At3g56940	0.63
2345	2346	delta-1-pyrroline-5-carboxylate synthetase	0	At3g55610	0.69
2347	2348	remorin	0	At2g45820	0.76
2349	2350	putative protein	0	At5g22460	0.48

2351	2352	putative lectin	0	At3g16530	0.43
2353	2354	putative protein	9E-29	At5g26260	0.52
2355	2356	peptidylprolyl isomerase ROC4	0	At3g62030	0.61
2357	2358	O-methyltransferase, putative	0	At1g21130	0.63
2359	2360	putative zinc finger protein	0	At4g38960	0.72
2361	2362	putative hydroxyproline-rich glycoprotein	1E-173	At1g13930	0.58
2363	2364	putative protein 1 photosystem II oxygen-evolving complex	0	At3g50820	0.65
2365	2366	hypothetical protein	0	At1g66700	0.63
2367	2368	unknown protein	0	At1g52870	0.43
2369	2370	heat shock protein 90	0	At5g56010	0.75
2371	2372	Overlap with bases 87,142-90,425 of 'IGF' BAC clone F9K20, accession	1E-115	At1g78570	0.63
2373	2374	phosphoglycerate kinase, putative	1E-120	At3g12780	0.73
2375	2376	putative lectin	1E-25	At3g16400	0.4
2377	2378	profilin 2	0	At4g29350	0.77
2379	2380	HSP associated protein like	5E-16	At4g22670	0.75
2381	2382	putative cell division control protein, cdc2 kinase	1E-75	At1g20930	0.72
2383	2384	putative protein	1E-107	At5g08050	0.65
2385	2386	ribosomal protein S27	0	At5g47930	0.77
2387	2388	vacuolar H+-transporting ATPase 16K chain	0	At4g34720	0.76
2389	2390	expansin At-EXP5	3E-82	At3g29030	0.52
2391	2392	similar to cold acclimation protein WCOR413 [Triticum aestivum]	0	At2g15970	0.74
2393	2394	chloroplast membrane protein (ALBINO3)	1E-159	At2g28800	0.72
2395	2396	putative thioredoxin	1E-102	At1g08570	0.55
2397	2398	unknown protein	0	At1g08380	0.65
2399	2400	hypothetical protein	0	At1g07180	0.53
2401	2402	putative flavonol sulfotransferase	0	At1g74090	0.69
2403	2404	possible apospory-associated like protein	0	At4g25900	0.71
2405	2406	glycolate oxidase, putative	0	At3g14420	0.71
2407	2408	putative peroxidase ATP2a	0	At2g37130	0.75
2409	2410	putative protein	1E-154	At4g21860	0.75
2411	2412	hydroxypyruvate reductase (HPR)	0	At1g68010	0.74
2413	2414	photosystem I reaction centre subunit psaN precursor (PSI-N)	0	At5g64040	0.49
2415	2416	plastid ribosomal protein S6, putative	0	At1g64510	0.6
2417	2418	methylenetetrahydrofolate reductase MTHFR1	0	At3g59970	0.72
2419	2420	putative photosystem I reaction center subunit II precursor	0	At1g03130	0.55
2421	2422	unknown protein	0	At3g10940	0.64
2423	2424	fumarate hydratase	0	At5g50950	0.43
2425	2426	Lil3 protein	0	At5g47110	0.73
2427	2428	homeobox gene ATH1	0	At4g32980	0.76
2429	2430	putative lectin	3E-20	At3g16390	0.43
2431	2432	COP1-interacting protein 7 (CIP7)	1E-07	At4g27430	0.67
2433	2434	putative acyl-CoA synthetase	0	At2g47240	0.51
2435	2436	unknown protein	0	At2g01590	0.68
2437	2438	hydroxymethyltransferase	0	At4g13930	0.72
2439	2440	hypothetical protein	1E-164	At1g69490	0.27
2441	2442	SNF1 related protein kinase (ATSRPK1)	1E-170	At3g23000	0.49
2443	2444	mevalonate diphosphate decarboxylase	6E-68	At2g38700	0.71
2445	2446	putative flavonol sulfotransferase	0	At1g74090	0.69
2447	2448	protein phosphatase 2C (AtP2C-HA)	0	At1g72770	0.59
2449	2450	cinnamoyl-CoA reductase - like protein	0	At4g30470	0.72
2451	2452	O-methyltransferase - like protein	0	At4g35160	0.5

2453	2454	pyruvate dehydrogenase E1 alpha subunit	0	At1g01090	0.77
2455	2456	putative chlorophyll A-B binding protein	0	At3g27690	0.49
2457	2458	putative UDP-N-acetylglucosamine pyrophosphorylase	0	At2g35020	0.69
2459	2460	putative protein	1E-121	At4g05590	0.75
2461	2462	Ca2+-dependent membrane-binding protein annexin	0	At1g35720	0.41
2463	2464	hypothetical protein	0	At2g35760	0.51
2465	2466	hypothetical protein	2E-15	At1g18840	0.71
2467	2468	hypothetical protein	0	At1g51140	0.53
2469	2470	aromatic amino-acid decarboxylase - like protein	0	At4g28680	0.73
2471	2472	unknown protein	3E-72	At2g35830	0.49
2473	2474	hypothetical protein	0	At1g78690	0.66
2475	2476	putative elongation factor P (EF-P)	0	At3g08740	0.74
2477	2478	unknown protein	0	At1g22750	0.76
2479	2480	putative protein	0	At3g63160	0.45
2481	2482	unknown protein	1E-150	At3g26510	0.55
2483	2484	aldo/keto reductase-like protein	0	At5g53580	0.69
2485	2486	glycine decarboxylase complex H-protein	0	At2g35370	0.53
2487	2488	thioredoxin (clone GIF1) (pir||S58118)	3E-14	At5g42980	0.53
2489	2490	putative protein	1E-93	At4g28020	0.52
2491	2492	hypothetical protein	0	At1g18870	0.71
2493	2494	vegetative storage protein Vsp2	0	At5g24770	0.43
2495	2496	putative protein	3E-75	At4g17560	0.66
2497	2498	NBD-like protein (gb|AAD20643.1)	0E+00	At5g44110	0.58
2499	2500	photosystem I subunit V precursor, putative	1E-119	At1g55670	0.56
2501	2502	putative thaumatin	2E-36	At2g28790	0.64
2503	2504	hyoscyamine 6-dioxygenase hydroxylase, putative	0	At1g35190	0.71
2505	2506	H-protein promoter binding factor-like protein	0	At5g62430	0.51
2507	2508	putative protein	0	At4g04840	0.52
2509	2510	endo-xyloglucan transferase - like protein	0	At4g37800	0.68
2511	2512	vitamine c-2	0	At4g26850	0.33
2513	2514	hypothetical protein	0	At3g12340	0.69
2515	2516	putative acetone-cyanohydrin lyase	0	At2g23610	0.68
2517	2518	putative transcription factor	0	At1g71030	0.36
2519	2520	hypothetical protein	1E-128	At1g19000	0.74
2521	2522	putative xyloglucan endo-transglycosylase	7E-27	At2g36870	0.4
2523	2524	hypothetical protein	3E-51	At1g58080	0.77
2525	2526	putative protein	1E-167	At5g36800	0.65
2527	2528	putative protein	1E-157	At4g30530	0.65
2529	2530	cinnamyl-alcohol dehydrogenase ELI3-1	0	At4g37980	0.54
2531	2532	putative CONSTANS-like B-box zinc finger protein	0	At2g47890	0.72
2533	2534	unknown protein	1E-123	At1g53480	0.6
2535	2536	protein phosphatase 2C-like protein	2E-55	At4g28400	0.72
2537	2538	putative protein	0	At5g60680	0.57
2539	2540	farnesyl-pyrophosphate synthetase FPS2	0	At4g17190	0.76
2541	2542	soluble inorganic pyrophosphatase, putative	0	At1g01050	0.59
2543	2544	putative nematode-resistance protein	1E-117	At2g40000	0.34
2545	2546	putative AP2 domain transcription factor	0	At2g23340	0.74
2547	2548	putative myo-inositol monophosphatase	3E-17	At3g02870	0.6
2549	2550	putative isoamylase	0	At1g03310	0.74
2551	2552	phosphate transporter (AtPT2)	0	At2g38940	0.76
2553	2554	putative disease resistance response protein	0	At4g11190	0.68
2555	2556	unknown protein	0	At2g45600	0.55

2557	2558	peroxidase ATP13a	0	At5g17820	0.7
2559	2560	unknown protein	0	At1g26920	0.74
2561	2562	putative mitochondrial carrier protein	0	At2g47490	0.69
2563	2564	actin depolymerizing factor 3 - like protein	1E-136	At5g59880	0.64
2565	2566	putative protein transport protein SEC23	1E-149	At2g21630	0.73
2567	2568	unknown protein	2E-30	At2g44310	0.74
2569	2570	putative protein	0	At4g21570	0.69
2571	2572	putative steroid binding protein	0	At2g24940	0.57
2573	2574	putative lipid transfer protein	0	At2g15050	0.49
2575	2576	hypothetical protein	0	At4g15510	0.75
2577	2578	unknown protein	3E-47	At3g25690	0.56
2579	2580	40S ribosomal protein S19 - like	0	At5g28060	0.73
2581	2582	putative auxin-regulated protein	0	At2g21210	0.56
2583	2584	unknown protein	0	At1g19350	0.71
2585	2586	unknown protein	1E-136	At1g07700	0.71
2587	2588	50S ribosomal protein L27	0E+00	At5g40950	0.7
2589	2590	unknown protein	1E-105	At2g46540	0.69
2591	2592	ATP-sulfurylase	0	At4g14680	0.72
2593	2594	hypothetical protein	1E-107	At3g18890	0.64
2595	2596	putative protein	0	At3g59780	0.62
2597	2598	cytochrome P450 monooxygenase - like protein	0	At4g37410	0.56
2599	2600	hypothetical protein	2E-86	At1g61890	0.36
2601	2602	ubiquitin-conjugating enzyme, putative	0	At3g20060	0.66
2603	2604	hypothetical protein	0	At1g20810	0.74
2605	2606	hypothetical protein	0	At2g15020	0.45
2607	2608	unknown protein	0	At1g55480	0.52
2609	2610	UDP glucose:flavonoid 3-o-glucosyltransferase -like protein	0	At5g17050	0.56
2611	2612	hypothetical protein	0	At3g23670	0.69
2613	2614	putative protein	0	At4g34920	0.69
2615	2616	unknown protein	1E-100	At2g36630	0.71
2617	2618	unknown protein	6E-94	At1g56580	0.63
2619	2620	HSR201 like protein	0	At4g15390	0.75
2621	2622	heme oxygenase 1 (HO1)	0	At2g26670	0.74
2623	2624	putative beta-glucosidase	0	At4g27820	0.46
2625	2626	unknown protein	1E-122	At1g68440	0.45
2627	2628	predicted protein	0	At4g22820	0.54
2629	2630	putative kinesin heavy chain	0	At2g22610	0.72
2631	2632	putative protein	0	At4g27860	0.61
2633	2634	unknown protein	0	At2g37240	0.76
2635	2636	unknown protein	0	At1g30070	0.76
2637	2638	WD-repeat protein -like protein	0	At4g33270	0.57
2639	2640	unknown protein	0	At1g32220	0.6
2641	2642	hypothetical protein	0	At4g22920	0.73
2643	2644	putative amino acid transporter protein	0	At3g11900	0.67
2645	2646	endo-beta-1,4-glucanase, putative	0	At1g64390	0.5
2647	2648	hypothetical protein	0	At1g18060	0.6
2649	2650	hypothetical protein	1E-114	At4g39820	0.7
2651	2652	putative protein	1E-62	At5g27290	0.6
2653	2654	putative protein	1E-133	At3g48200	0.46
2655	2656	hypothetical protein	1E-173	At1g64500	0.51
2657	2658	putative ribonuclease, RNS2	0	At2g39780	0.6
2659	2660	thioredoxin f1	0	At3g02730	0.59

2661	2662	unknown protein	0	At2g20670	0.67
2663	2664	cytochrome P450-like protein	0	At5g48000	0.45
2665	2666	subtilisin proteinase - like	1E-105	At4g21650	0.31
2667	2668	photoassimilate-responsive protein PAR-1b -like protein	0E+00	At3g54040	0.76
2669	2670	putative dTDP-glucose 4-6-dehydratase	0	At2g27860	0.45
2671	2672	hypothetical protein	0	At1g51700	0.43
2673	2674	early light-induced protein	0	At3g22840	0.65
2675	2676	hypothetical protein	0	At1g32060	0.42
2677	2678	unknown protein	0	At2g34860	0.69
2679	2680	peroxidase ATP3a (emb|CAA67340.1)	4E-10	At5g64100	0.49
2681	2682	putative protein	0	At5g06770	0.67
2683	2684	hypothetical protein	0	At2g16860	0.57
2685	2686	annexin	0	At5g65020	0.61
2687	2688	thioredoxin, putative	0	At1g50320	0.63
2689	2690	putative protein	0	At5g17360	0.66
2691	2692	nucleoside diphosphate kinase 3 (ndpk3)	0	At4g11010	0.76
2693	2694	unknown protein	0	At5g62550	0.64
2695	2696	putative protein	0	At4g12000	0.62
2697	2698	cell division protease FtsH, putative	0	At1g06430	0.65
2699	2700	unknown protein	0	At1g74880	0.41
2701	2702	putative protein	0	At5g56540	0.61
2703	2704	unknown protein	0	At1g68780	0.61
2705	2706	mipC protein - like (aquaporin)	0	At5g60660	0.64
2707	2708	Oxygen-evolving enhancer protein 3 precursor - like protein	0	At4g05180	0.64
2709	2710	cytochrome P450, putative	0	At3g26180	0.74
2711	2712	putative protein	1E-126	At5g22210	0.74
2741	2742	unknown protein		At1g45200	3.91
2743	2744	unknown protein, putative protease inhibitor		At5g43580	2.58
2745	2746	putative protein		At5g03540	2.21
2747	2748	putative WD repeat protein		At3g15880	2.38
2749	2750	putative protease inhibitor Dr4		At1g73330	10.30
2751	2752	putative auxin regulated protein		At2g46690	2.86
2753	2754	translation initiation factor like protein		At5g54940	2.15
2755		pseudogene		At2g04110	2.07

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CLAIMS

1. A method to alter one or more plant characteristics, said method comprising modifying, in a plant, expression of one or more nucleic acids and/or modifying level and/or activity of one or more proteins, which nucleic acids and/or proteins are essentially similar to any one of SEQ ID NO 1 to 2755, and wherein said one or more plant characteristics are altered relative to corresponding wild type plants.

2. A method according to claim 1, wherein said altered plant characteristic is selected from any one or more of the following: altered development, altered growth, increased yield and/or biomass, enhanced survival capacity, enhanced stress tolerance, altered plant architecture, altered plant physiology, altered plant biochemistry, altered metabolism, altered DNA synthesis, altered DNA modification, altered endoreduplication, altered cell cycle, altered cell wall biogenesis, altered transcription regulation, altered signal transduction, altered storage lipid mobilization and/or altered photosynthesis, each relative to corresponding wild type plants.

3. A method according to claim 2, wherein said altered metabolism comprises altered nitrogen and/or altered carbon metabolism.

4. A method according to claim 2, wherein said increased yield and/or biomass, comprises increased seed yield.

5. A recombinant nucleic acid comprising:

(a) one or more nucleic acid sequences essentially similar to any one of SEQ ID NO 1 to 2755 or the complement strand thereof; optionally operably linked to

(b) a regulatory sequence, and optionally operably linked to

(c) a transcription termination sequence

6. A recombinant nucleic acid according to claim 5, wherein said regulatory sequence is a plant-expressible promoter.

7. A recombinant nucleic acid according to claims 6, wherein said plant-expressible promoter is any one of the promoters listed in Table I, II, III or IV.

8. A method for making a transgenic plant or plant cell having one or more altered plant characteristics when compared to the corresponding wild-type characteristics, said method comprising introduction of a recombinant nucleic acid according to claim 5, 6 or 7 into said plant or plant cell.

9. A method according to claim 8, wherein said recombinant nucleic acid is stably integrated into the genome of said plant .

10. A method according to any of claims 1 to 4 or 8 or 9, comprising overexpression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755.

11. A method according to any of claims 1 to 4 or 8 or 9, comprising downregulation of expression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755.

12. A transgenic plant having one or more altered characteristics when compared to the corresponding wild-type plant, characterized in that said plant has modified expression of one or more nucleic acids and/or modified level and/or activity of one or more proteins, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

13. A transgenic plant obtainable by a method according to any of claims 1 to 4 or 8 to 11.

14. A transgenic plant comprising an isolated nucleic acid and/or protein sequence essentially similar to any one of SEQ ID NO 1 to 2755.

15. An ancestor, progeny, or any plant part, particularly a harvestable part, of a transgenic plant of claim 12 or 14.

16. A host cell having one or more altered characteristics when compared to the corresponding wild-type host cell, characterized in that said host cell has modified expression of one or more nucleic acids and/or modified level and/or activity of one or more proteins, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

17. Use of a nucleic acid sequence or protein essentially similar to any one of SEQ ID NO 1 to 2755, for altering one or more plant characteristics.

18. A method for the production of plants with one or more altered characteristics when

compared to the corresponding wild-type plants, which method comprises the use of a nucleic acid sequence essentially similar to any of SEQ ID NO 1 to 2755 in marker assisted breeding.

19. A method for the production of plants with one or more altered characteristics when compared to the corresponding wild-type plants, which method comprises the use of a nucleic acid sequence essentially similar to any of SEQ ID NO 1 to 2755 in conventional breeding.

20. A plant obtainable by the methods according to claim 18 or 20.

21. Use of a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755 as a growth regulator.

22. A growth regulating composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

23. A method for the production of a growth regulator, comprising the production of a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

24. Method for the production of enzymes and/or pharmaceuticals, which method comprises modifying expression of a nucleic acid, and/or modifying level and/or activity of a protein, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

25. Use of plants according to claim 11 to 14, for the production of enzymes and/or pharmaceuticals.

26. Enzymes and pharmaceuticals produced according to the method of claim 24.

27. Use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755, as a therapeutic agent, a diagnostic means, a kit or plant effective agent.

28. A therapeutic composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

29. A diagnostic composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

30. A kit comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

31. A plant effective agent comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

32. Method for the manufacturing of a therapeutic composition, a diagnostic composition, a kit or a plant effective agent, comprising the production of a sequence essentially similar to any one of SEQ ID NO 1 to 2755.

33. A food product derived from a plant or host cell according to any one of claim 12 to 16.

34. Use of a food product according to claim 33 in animal feed or food.

35. Method for the production of a food or feed product, which method comprises modifying expression of a nucleic acid, and/or modifying level and/or activity of a protein, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

36. Use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755 as a positive or negative selectable marker during transformation of cells or tissues or during cell procedures.

37. Use according to claim 36, wherein said cell is derived from a plant, animal, bacterium, fungus, yeast, insect, algae.

38. An isolated nucleic acid comprising one or more of the regulatory elements upstream of the startcodon of any of the nucleic acids represented by SEQ ID NO 1 to 2755.

39. An isolated nucleic acid according to claim 38, wherein said regulatory element is the natural promoter of any one of said nucleic acids represented by SEQ ID NO 1 to 2755.

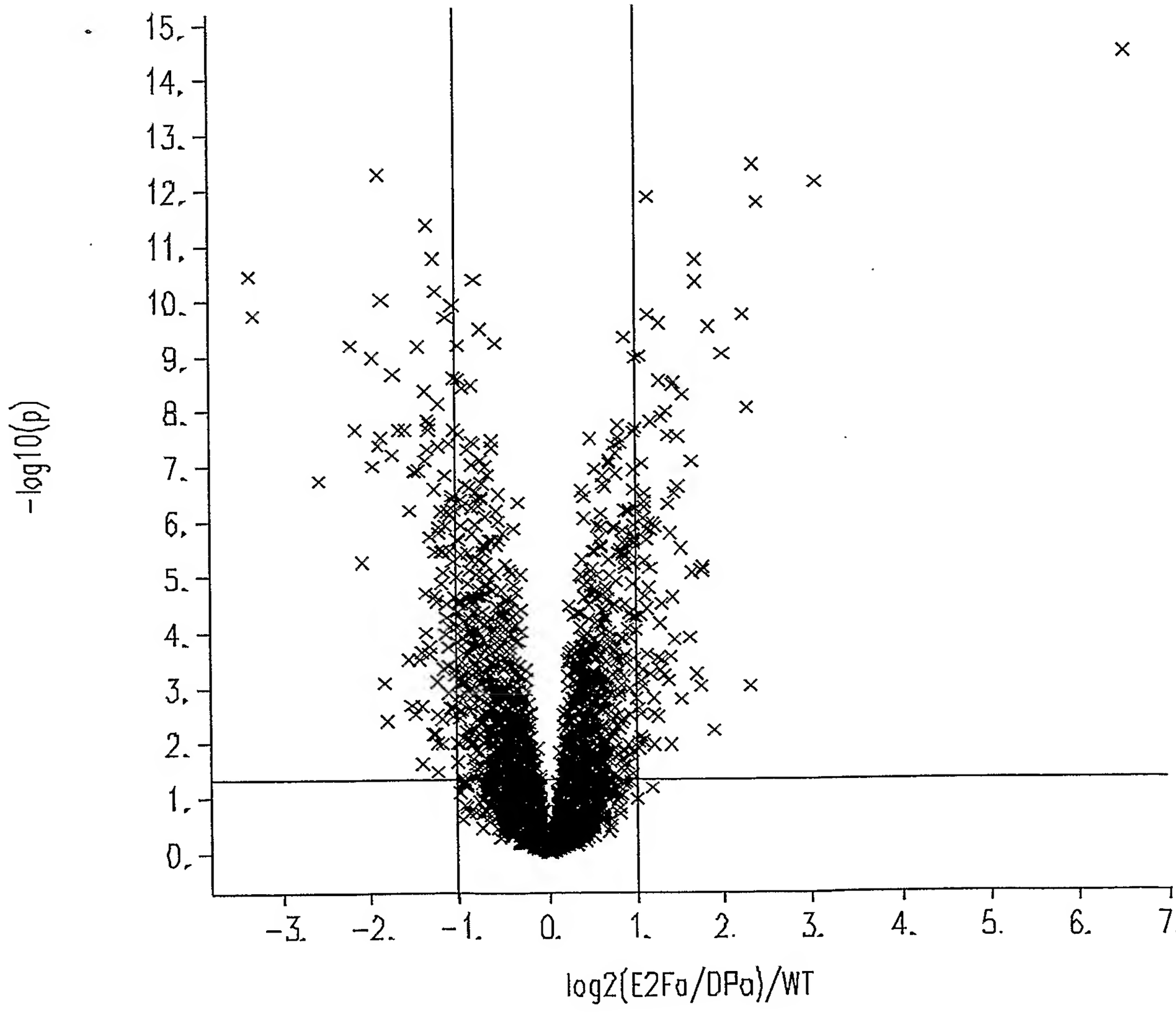

15.
14.
13.
12.
11.
10.
9.
8.
7.
6.
5.
4.
3.
2.
1.
0.
−log10(p)
−3.
−2.
−1.
0.
1.
2.
3.
4.
5.
6.
7.
log2(E2Fa/DPa)/WT

FIGURE 1

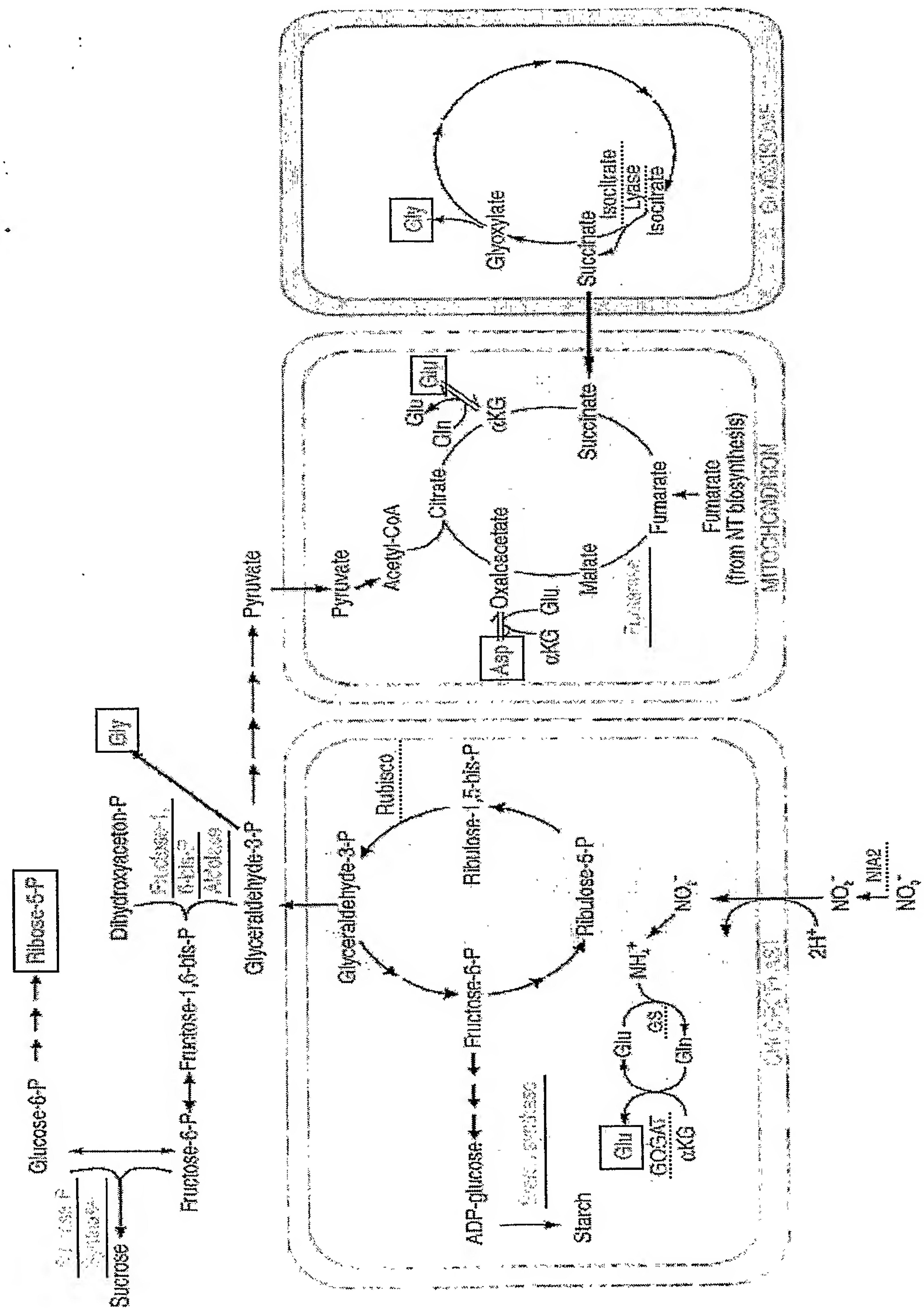
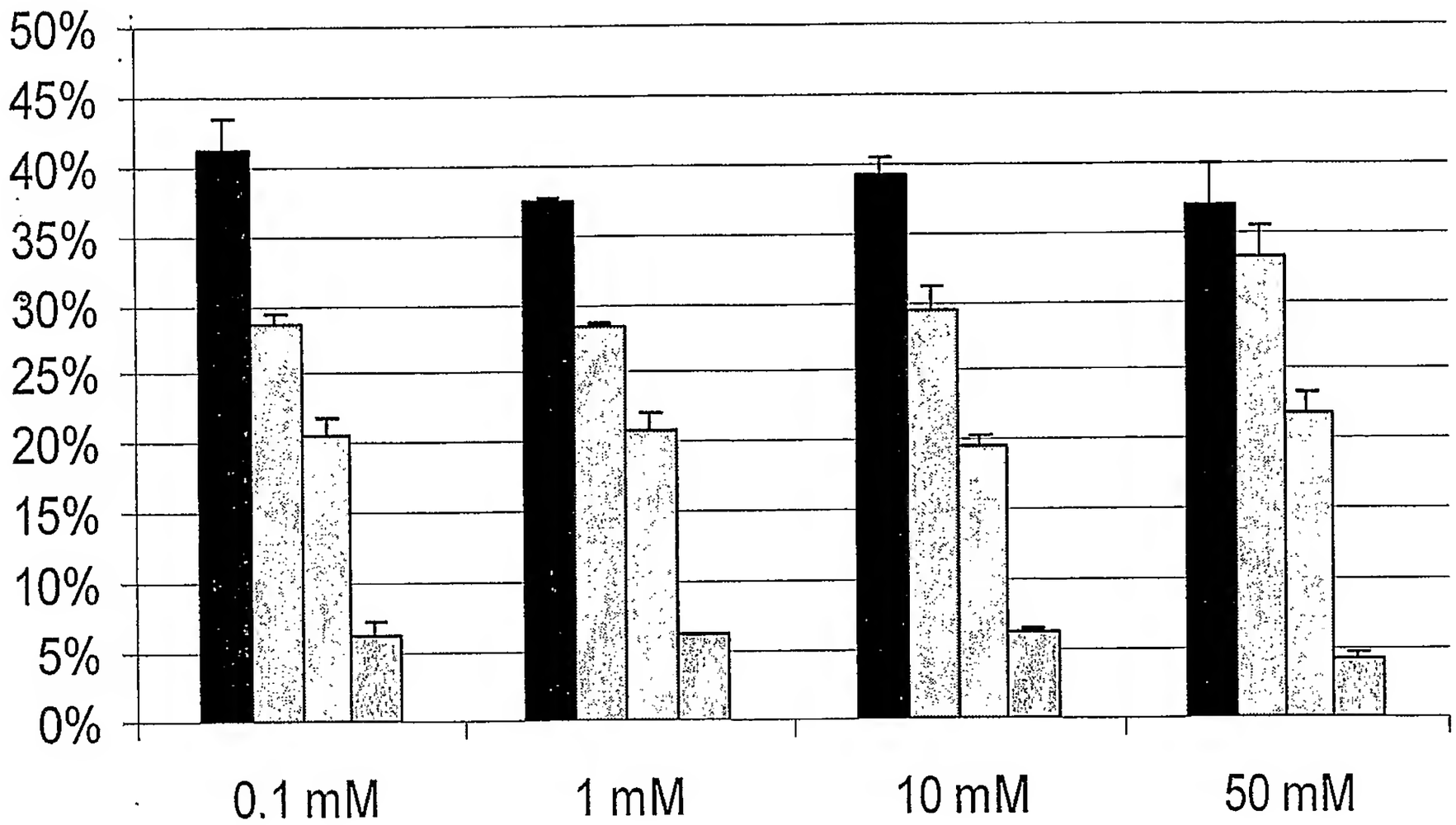

Gly
Glyoxylate
Succinate
Isocitrate
Lyase
Isocitrate
Glu
Glu
Gln
αKG
Succinate
Citrate
Acetyl-CoA
Pyruvate
Pyruvate
Oxalacetate
Asp
αKG
Glu
Malate
Fumarate
Fumarate
(from NT biosynthesis)
MITOCHONDRION
Gly
Dihydroxyaceton-P
Glyceraldehyde-3-P
Fructose-1,6-bis-P
Ribose-5-P
Glucose-6-P
Fructose-6-P
Sucrose
Rubisco
Glyceraldehyde-3-P
Ribulose-1,5-bis-P
Ribulose-5-P
Fructose-6-P
ADP-glucose
Starch
NH4+
NO2-
Glu
GS
Gln
Glu
GOGAT
αKG
2H+
NO2-
NIA2
NO3-

FIGURE 2

A


50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
0.1 mM
1 mM
10 mM
50 mM

B

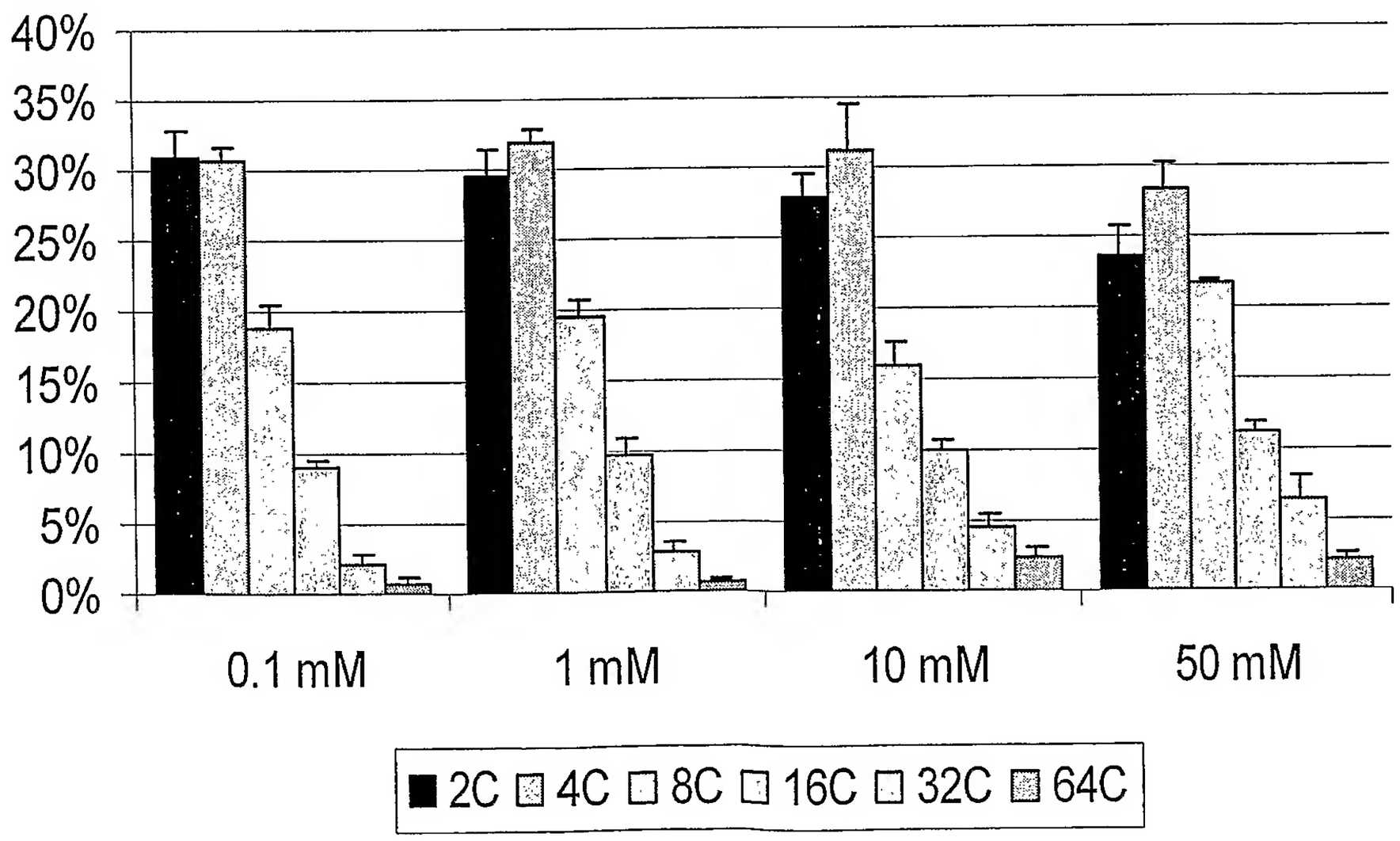

40%
35%
30%
25%
20%
15%
10%
5%
0%
0.1 mM
1 mM
10 mM
50 mM
2C
4C
8C
16C
32C
64C

FIGURE 3

MATDB - entry At1g57680 from contig t8l23 mips
(Chromosome 1 / BAC clone T8L23 / sequence database accession EMBL:AC079733)

Type: gene/protein
Code: At1g57680
Old code: T8L23_15
Title: putative protein
Contig: t8l23
Position: 53392-54480 (C)

Notes

•

Classification

- known protein

Functional Category

- UNCLASSIFIED PROTEINS

TargetP prediction

- Targeted to secretory pathway
- TargetP score: 0.968
- TargetP reliability class: 2
- Probable signal sequence length: -

TMHMM transmembrane prediction

- Very likely to be a transmembrane protein (or have a signal peptide) (Exp number of AA in TMHs: 110)
- A transmembrane region could actually be a signal peptide (Exp number, first 60 AAs: 21)
- Orientation of N-terminal: external side (probability: 0.9)
- Transmembrane regions:
  - 40-62
  - 83-100
  - 138-160
  - 181-203
  - 213-235

EMBL

- AY072149

mRNA matches: 1 found

Arabidopsis ESTs

- found **10**
  AA585779; AI992654; AI998042; AV518701; AV538415; AV538372; AV541088; AV550688; AV550640; AV554579;

FIGURE 4

Full report

- Full report includes FST matches and external annotation... slow.

Protein properties

PEDANT and Interpro data are being recalculated. To access old PEDANT data, use the link in the left frame, but be aware that some protein sequences have been changed due to update of gene models based on cDNA data and PEDANT data may be outdated.

Click here to submit new information about this entry

FIGURE 4 (contin.)

A. thaliana - contig t8l23 - entry At1g57680 mips

```
>P1;At1g57680
putative protein
MPLTKLVPDA FGVVTICLVA LLVLLGLLCI AYSFYFQSHV RKQGYIQLGY FSGPWIIRIT
FILFAIWWAV GEIFRLSLLR RHRRLLSGLD LRWQENVCKW YIVSNLGFAE PCLFLTLMFL
LRAPLKMESG ALSGKWNRDT AGYIILYCLP MLALQLAVVL SESRLNGGSG SYVKLPHDFT
RTYSRVIIDH DEVALCTYPL LSTILLGVFA AVLTAYLFWL GRQILKLVIN KRLQKRVYTL
IFSVSSFLPL RIVMLCLSVL TAADKIIFEA LSFLAFLSLF CFCVVSICLL VYFPVSDSMA
LRGLRDTDDE DTAVTEERSG ALLLAPNSSQ TDEGLSLRGR RDSGSSTQER YVELSLFLEA
EN*
C; Length 362 aa
C; Sequence At1g57680 was extracted from t8l23
C; Fragment (54480-53392(C))
```


FIGURE 4 (contin.)

A. thaliana - contig t8l23 - coordinates: 53392-54480 (C) mips

```
   1 ATGCCCCTGA CAAAATTAGT TCCCGATGCA TTCGGCGTTG TGACGATATG TCTAGTCGCT
  61 CTGCTAGTTC TTTTGGGTCT CCTTTGCATC GCTTACTCGT TCTATTTCCA GTCTCACGTT
 121 CGTAAGCAAG GCTATATTCA ACTTGGTTAC TTCAGTGGTC CCTGGATTAT CCGAATCACT
 181 TTCATTCTCT TTGCTATCTG GTGGGCTGTT GGTGAGATTT TTCGATTGAG TTTGTTGAGG
 241 CGTCACAGAA GGTTGTTGAG TGGGTTGGAT CTGAGATGGC AAGAAAACGT TTGCAAGTGG
 301 TACATCGTTT CCAATCTAGG ATTTGCGGAG CCTTGTCTCT TTCTGACTCT CATGTTTCTT
 361 CTGCGTGCTC CCTTGAAGAT GGAATCAGGG GCTTTGAGCG GAAAATGGAA CAGGGACACA
 421 GCAGGTTATA TTATTCTTTA TTGTCTCCCG ATGCTTGCTC TTCAACTTGC GGTTGTGTTG
 481 TCCGAGTCAC GCCTAAATGG TGGTAGTGGC TCTTATGTAA AGCTGCCACA CGACTTCACA
 541 AGAACGTATT CCCGAGTTAT TATTGATCAC GACGAGGTGG CCTTATGCAC ATATCCTCTA
 601 CTGAGTACCA TCCTTCTTGG TGTGTTTGCA GCCGTCCTAA CAGCTTACTT GTTCTGGCTT
 661 GGAAGGCAGA TACTGAAACT TGTCATTAAC AAGCGTTTAC AGAAGAGAGT ATACACTTTG
 721 ATATTCTCGG TCTCGAGTTT CCTTCCATTA AGGATTGTTA TGCTCTGTTT GTCGGTTCTC
 781 ACAGCAGCAG ACAAGATTAT ATTCGAAGCC CTTTCTTTCT TGGCCTTCCT CTCCCTCTTC
 841 TGCTTTTGCG TGGTATCCAT CTGCTTGCTT GTCTACTTCC CGGTTTCAGA TTCCATGGCC
 901 CTGAGAGGTC TAAGAGACAC AGATGATGAG GATACGGCTG TGACCGAAGA ACGCAGTGGT
 961 GCTCTGTTAC TTGCACCAAA CTCTTCACAA ACTGATGAGG GATTGAGCTT AAGAGGTCGG
1021 AGAGACTCGG GATCGTCTAC ACAGGAGAGG TATGTGGAAC TCAGCCTATT TCTGGAAGCT
1081 GAGAACTAA
```

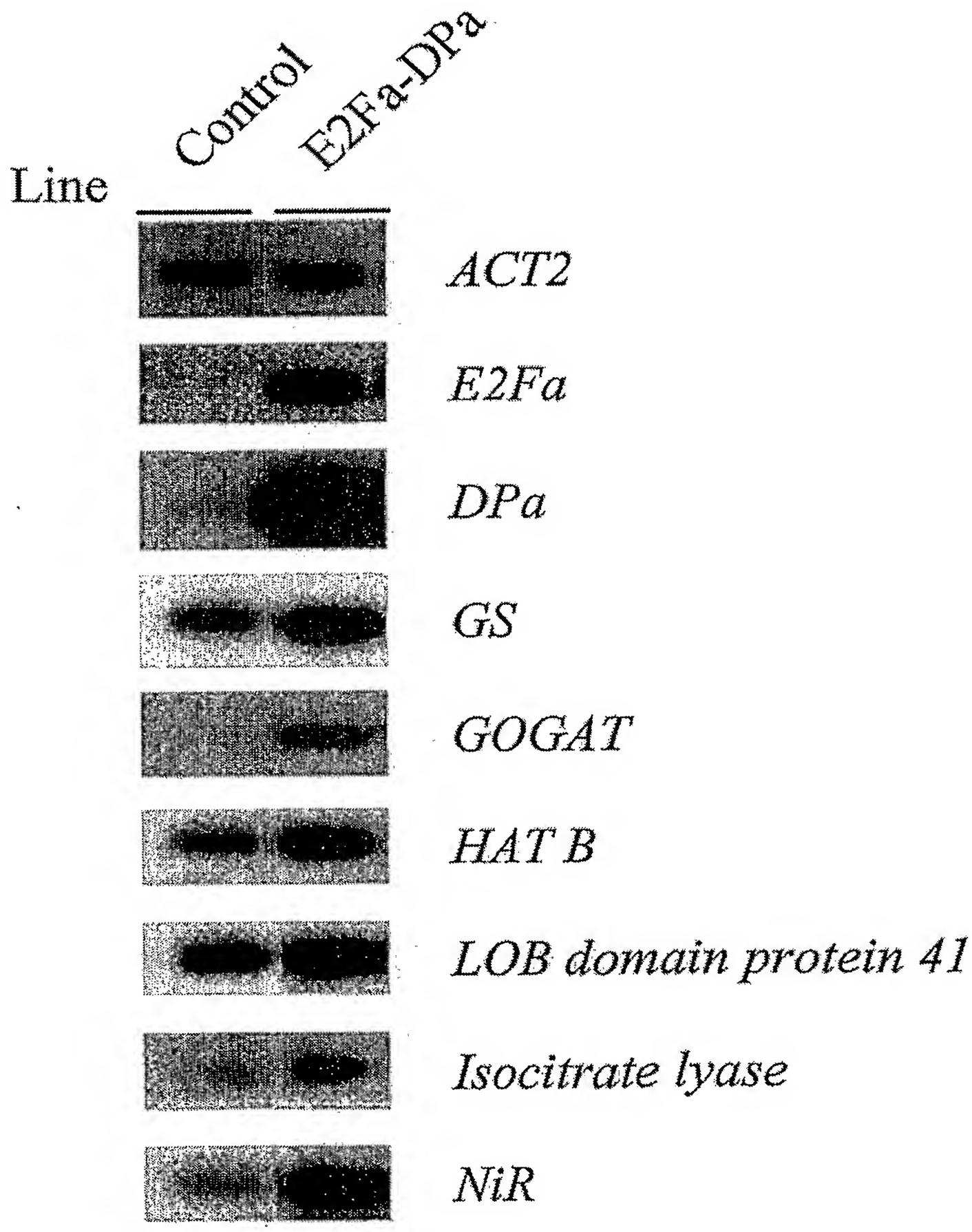

FIGURE 4 (contin.)

A. thaliana - contig t8l23 - coordinates: 53392-54480 (C) mips

```
    1 gtattatcta ttgagattct gtgtttaaag gttatgactt ggctttatgt atttcaatat
   61 tgttattgat ttgtgtgtta atcatcttaa tcttcaaggt tgctttagtt ttgagaaagg
  121 ttatgacttt ttaagatcta gggttaagat ggtttgatag gtctttgttt caaatttttg
  181 gtatatttgg taagtttttg attatttgtt gttttgttta gtttatgtag gtaacacgca
  241 tatcaagtgt taaagagtca agatcacaaa aagttctatc gggtgatctg ggctgctttc
  301 ttttgtaatc taattgcaga aactttgctc tgacttggat agcttcgtaa aaggttcaat
  361 ctttctccgt ttttcatcaa tgagtagtaa ctaatctgga aatttgttgg gagagaaagg
  421 gcacattgca ctgctattgc tagagaacgt ttctgcatcc atgctggtag agagcatgcg
  481 tggatactgt gttttgggtg ATGCCCCTGA CAAAATTAGT TCCCGATGCA TTCGGCGTTG
  541 TGACGATATG TCTAGTCGCT CTGCTAGTTC TTTTGGGTCT CCTTTGCATC GCTTACTCGT
  601 TCTATTTCCA GTCTCACGTT CGTAAGCAAG GCTATATTCA ACTTGGTTAC TTCAGTGGTC
  661 CCTGGATTAT CCGAATCACT TTCATTCTCT TTGCTATCTG GTGGGCTGTT GGTGAGATTT
  721 TTCGATTGAG TTTGTTGAGG CGTCACAGAA GGTTGTTGAG TGGGTTGGAT CTGAGATGGC
  781 AAGAAAACGT TTGCAAGTGG TACATCGTTT CCAATCTAGG ATTTGCGGAG CCTTGTCTCT
  841 TTCTGACTCT CATGTTTCTT CTGCGTGCTC CCTTGAAGAT GGAATCAGGG GCTTTGAGCG
  901 GAAAATGGAA CAGGGACACA GCAGGTTATA TTATTCTTTA TTGTCTCCCG ATGCTTGCTC
  961 TTCAACTTGC GGTTGTGTTG TCCGAGTCAC GCCTAAATGG TGGTAGTGGC TCTTATGTAA
 1021 AGCTGCCACA CGACTTCACA AGAACGTATT CCCGAGTTAT TATTGATCAC GACGAGGTGG
 1081 CCTTATGCAC ATATCCTCTA CTGAGTACCA TCCTTCTTGG TGTGTTTGCA GCCGTCCTAA
 1141 CAGCTTACTT GTTCTGGCTT GGAAGGCAGA TACTGAAACT TGTCATTAAC AAGCGTTTAC
 1201 AGAAGAGAGT ATACACTTTG ATATTCTCGG TCTCGAGTTT CCTTCCATTA AGGATTGTTA
 1261 TGCTCTGTTT GTCGGTTCTC ACAGCAGCAG ACAAGATTAT ATTCGAAGCC CTTTCTTTCT
 1321 TGGCCTTCCT CTCCCTCTTC TGCTTTTGCG TGGTATCCAT CTGCTTGCTT GTCTACTTCC
 1381 CGGTTTCAGA TTCCATGGCC CTGAGAGGTC TAAGAGACAC AGATGATGAG GATACGGCTG
 1441 TGACCGAAGA ACGCAGTGGT GCTCTGTTAC TTGCACCAAA CTCTTCACAA ACTGATGAGG
 1501 GATTGAGCTT AAGAGGTCGG AGAGACTCGG GATCGTCTAC ACAGGAGAGG TATGTGGAAC
 1561 TCAGCCTATT TCTGGAAGCT GAGAACTAA a atcgccaaag gctgtttcta tttggctttt
 1621 ggcaatgtac agattcctgg tgaaaccagc agagagagag ggataaagag tttaagtatg
 1681 agaatatgtt tgcgcaaaaa aaggcatcat ttcagttttg tggcaaagac actttgactg
 1741 taaaggaggg ttttaagggg tttactcttg tgagggtttg ttgtttgaaa tgtttttctgc
 1801 ttgatggatc atatttttgt acctttatta tgtgatcaat tttgatttag aaaaaaaaaa
 1861 aaaaaacaat agccgtgaac atgagcatga ctttaaaaga taaatcagcc ttttaccctc
 1921 tattctttgg actcatgaac atgagacttc ataagaaaat tagagaaatt gtaccaagca
 1981 aaacagccat atagtgttct aaacttccat gtcaattcga cctagacaaa tacacttatg
 2041 acttcagaaa atttgacata attttaatat ttaaccaagt ttgtcaaga
```

Sequences of 5' leader, 3' trailer, and introns (when applicable) are printed in lowercase.

FIGURE 4 (contin.)


Line
Control
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ACT2
E2Fa
DPa
GS
GOGAT
HAT B
LOB domain protein 41
Isocitrate lyase
NiR

FIGURE 5

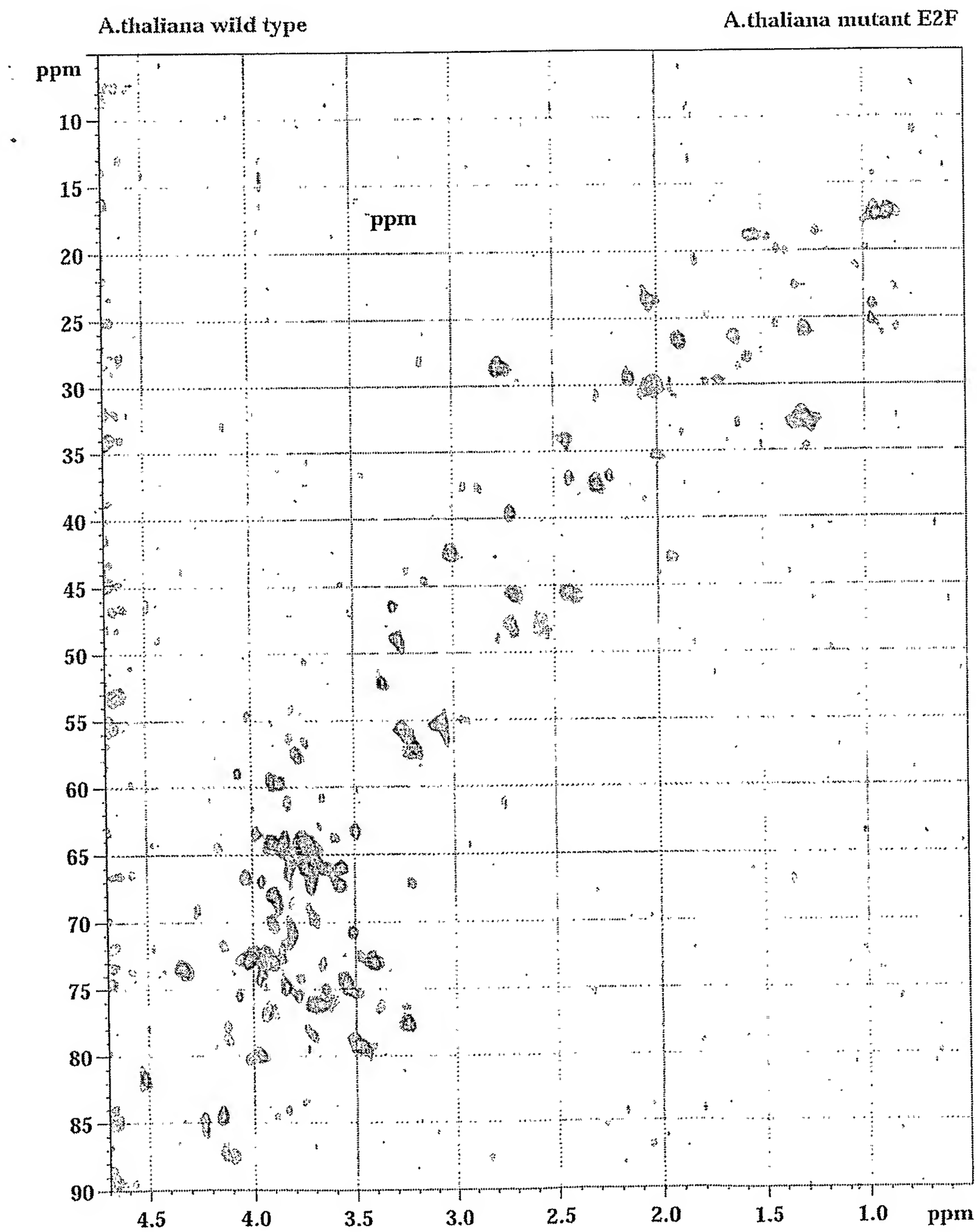
A.thaliana wild type
A.thaliana mutant E2F
ppm
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

FIGURE 6

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 April 2004 (29.04.2004)

PCT

(10) International Publication Number
WO 2004/035798 A3

(51) International Patent Classification[7]: C12N 15/82, C07K 14/415, A01H 1/04, 5/00, A61K 31/713, 38/16

(21) International Application Number: PCT/EP2003/011658

(22) International Filing Date: 20 October 2003 (20.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02079408.7 18 October 2002 (18.10.2002) EP

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(75) Inventors/Applicants *(for US only)*: **INZE, Dirk** [BE/BE]; Driesstraat 18, B-9310 Moorsel-Aalst (BE). **DE VEYLDER, Lieven** [BE/BE]; Josef Boddaertdreef 23, B-9031 Drongen (BE). **VLIEGHE, Kobe** [BE/BE]; Ter Weibroek 51, B-9880 Aalter (BE).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

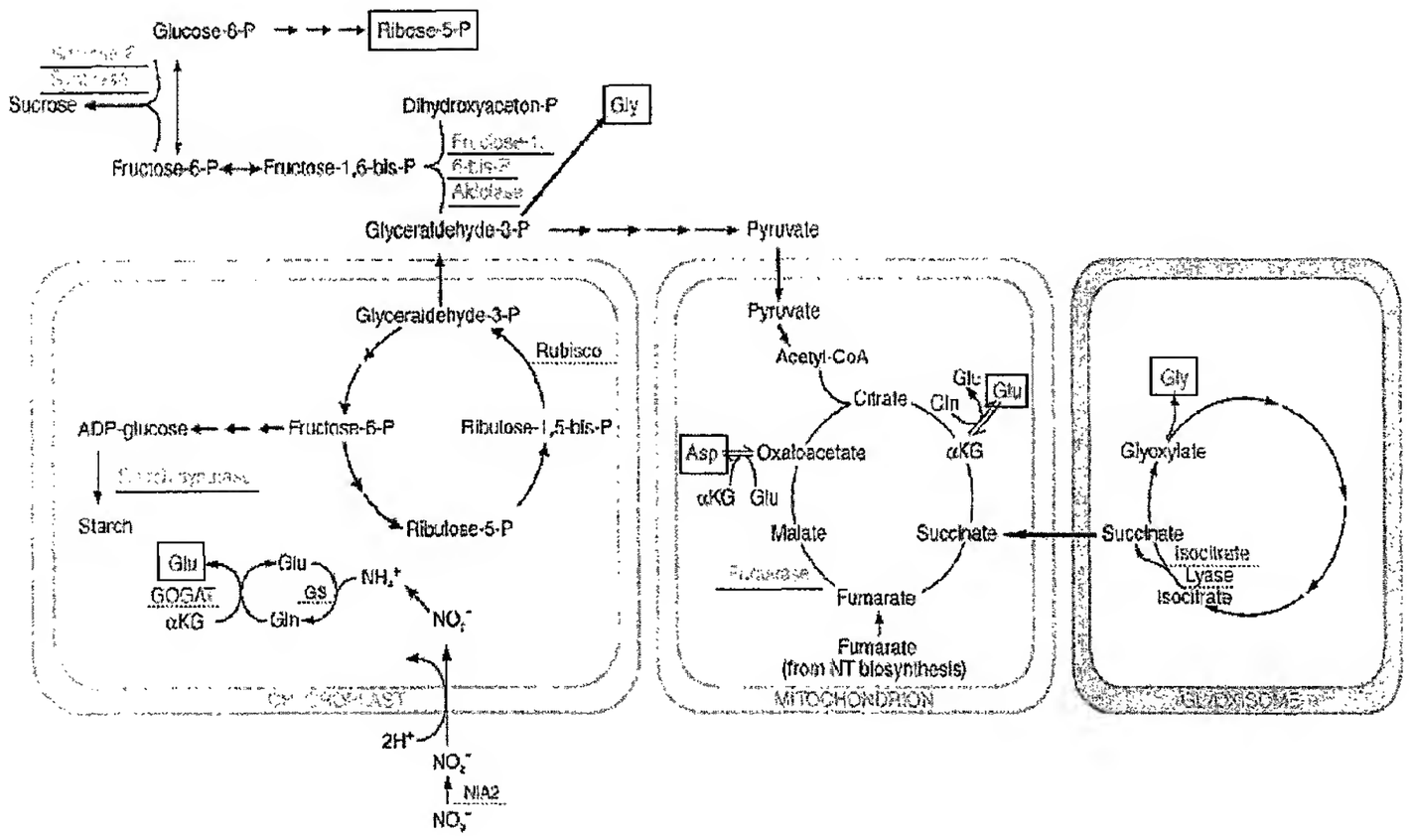
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 4 November 2004

[Continued on next page]

(54) Title: IDENTIFICATION OF E2F TARGET GENES AND USES THEREOF



(57) Abstract: The present invention concerns a method for altering characteristics of a plant. The invention describes the identification of genes that are upregulated or downregulated in transgenic plants overexpressing E2Fa/DPa and the use of such sequences to alter plant characteristics. A preferred way for altering characteristics of a plant comprises modifying expression of one or more nucleic acid sequences and or modifying level and/or activity of one or more proteins, which nucleic acids and/or proteins are essentially similar to any one or more of SEQ ID NO 1 to 2755. Some of the genes identified in the present invention have an E2Fa target consensus sequence in their 5' upstream region. The identified genes play a role in a variety of biological processes, such as DNA replication, cell wall biosynthesis, nitrogen and/or carbon metabolism, transcription factors etc.

WO 2004/035798 A3

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/11658

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C07K14/415 A01H1/04 A01H5/00 A61K31/713
A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 033 405 A (CERES INC) 6 September 2000 (2000-09-06) page 341, line 11 - line 13; sequences 72617,72618 claims 1-34 -----	1-16,30, 32,36,37
L	DATABASE EMBL [Online] 18 October 2000 (2000-10-18), ALEXANDROV N. ET AL.: "Arabidopsis thaliana DNA fragment SEQ ID NO:72617" XP002283128 Database accession no. AAC52840 L: document disclosing SEQ ID NO:72617 of patent EP 1 033 405 the whole document -----	1-16,30, 32,36,37
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2004	01. 09. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Blanco Urgoiti, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/11658

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L	DATABASE EMBL [Online] ALEXANDROV N. ET AL.: "Arabidopsis thaliana protein fragment SEQ ID NO:72618" XP002283129 Database accession no. AAG56488 L: document disclosing SEQ ID NO:72618 of patent EP 1 033 405 the whole document -----	1-16,30, 32,36,37
X	DATABASE EMBL [Online] XP002283411 Database accession no. AC079733 nts 53131-54759 protein-id:AAG50748.1 page 8 -----	5-11,16, 30,32, 36-39
X	VEYLDER DE L ET AL: "Control of proliferation, endoreduplication and differentiation by the Arabidopsis E2Fa-DPa transcription factor" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 21, no. 6, 15 March 2002 (2002-03-15), pages 1360-1368, XP002227182 ISSN: 0261-4189 cited in the application the whole document -----	1-4, 12-16,25
A	VANDEPOELE K ET AL: "Genome-wide analysis of core cell cycle genes in Arabidopsis" PLANT CELL, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, US, vol. 14, no. 4, April 2002 (2002-04), pages 903-916, XP002259203 ISSN: 1040-4651 cited in the application -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/11658

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 19 relates to an essentially biological process for the production of plants (Rule 39.1(ii) PCT)

2. [X] Claims Nos.: 26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-39 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claim 19 relates to an essentially biological process for the production of plants (Rule 39.1(ii) PCT)

Continuation of Box I.2

Claims Nos.: 26

Claim 26 refers to enzymes and/or pharmaceuticals produced using the transgenic plants of claims 12 to 14 without giving a true technical characterization. Moreover, no such compounds are defined the application. In consequence, the scope of said claim is ambiguous and vague, and itsr subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claim whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-39 (all partially)

Method to alter plant characteristics modifying the expression of the gene of SEQ ID NO:1 or the protein level of SEQ ID NO:2; the nucleic acid of SEQ ID NO:1; method for makeing a transgenic plant introducing the DNA of SEQ ID NO:1; transgenic plants; host cells; methods for plant breeding; the DNA of SEQ ID NO:1 or the protein of SEQ ID NO:2 comprised in growth regulators, in therapeutic/diagnostic compositions, in kits or in food products; use of SEQ ID NOs:1 or 2 as markers; regulatory elements and promoters of the DNA of SEQ ID NO:1.

Invention 2: claims 1-39 (all partially)

As invention 1 but related to the DNA of SEQ ID NO:3 and the polypeptide of SEQ ID NO:4.

Invention 3 to 1368: Claims 1-39 (all partially)

Invention 3 being as invention 1 but related to SEQ ID NOs:5 and 6;...invention 1368 being as invention 1 but related to SEQ ID NO:2755.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/11658

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1033405 A	06-09-2000	CA 2300692 A1	25-08-2000
		EP 1033405 A2	06-09-2000